Vol. 10 No. 3

PLANT PHYSIOLOGY

JULY, 1935

ACCUMULATION OF BORON BY RECIPROCALLY GRAFTED PLANTS

FRANK M. EATON AND GEORGE Y. BLAIR[1]

(WITH THREE FIGURES)

Introduction

The experimental data presented in this paper show that the amount of boron accumulated by plants may be materially influenced by the rootstock. The boron relations of agricultural plants have been found to be diverse and the characteristics of boron accumulation are in some measure differentiated from the accumulation characteristics of other elements. Certain facts in this regard, illustrated in the following three paragraphs and more extensively presented elsewhere (**1**), provide in a measure a background for the experimental results here to be considered.

An outstanding variability exists in the extent to which different plants take up boron. The boron concentrations resulting in entire plants, expressed in parts per million on the basis of dry weights, when grown in sand cultures maintained with a nutrient solution containing 5 p.p.m. of the element, were as follows: Navy beans 648, lima beans 515, wheat 453, pumpkin 291, lettuce 261, turnips 245, Canadian field peas 207, tomatoes 159, alfalfa 139, Acala cotton 123, asparagus 120, and carrots 69.

The distribution of boron throughout a plant is not uniform; it tends to accumulate in some parts more than in others. The leaves of rooted lemon cuttings grown in the cultures just mentioned contained 1232 p.p.m. of boron and the combined stems and roots 54 p.p.m. Young elms had 943 p.p.m. in their leaves and 22 p.p.m. in their stems; corn had 60 p.p.m. in the combined leaves, stalks, and roots and 16 p.p.m. in the grain; roses had 220 p.p.m. in their leaves and 20 p.p.m. in their stems. Twigs of boron-injured prune trees in an orchard planting contained 176 p.p.m. of boron in their leaves,

[1] Special acknowledgment is made of the indispensable analytical work carried out by V. P. SOKOLOFF and L. V. WILCOX.

412 p.p.m. in the bark, and 171 p.p.m. in the wood. Twigs of slightly injured lemon trees on the other hand contained 410 p.p.m. in their leaves, 42 p.p.m. in the bark, and but 9 p.p.m. in the wood. As shown by SCOFIELD and WILCOX (7), orange trees with 854 p.p.m. of boron in their leaves contained but 40 p.p.m. in the dried fruits.

Within particular plant parts differences in accumulation are likewise found. A sample of injured lemon leaves showing marginal burning and the characteristic yellowing of the leaf tissue farther removed from the veins had 47 p.p.m. of boron in the midveins and petioles, 438 p.p.m. in the green portion of the leaf laminae, 1060 p.p.m. in the yellow portions, and 1722 p.p.m. in the dead margins and apices. The bark at the enlarged nodes of the boron-injured prune twigs just referred to contained more boron than the bark of the internodes.

The facts that in most plants boron tends to accumulate in leaves, and in these at points farthest removed from the water-conducting tissue, suggest that it is carried to the leaves by the transpiration stream. The same facts also imply that boron as taken up and as moved to the leaves (probably as an inorganic radical) is highly mobile, but upon reaching the leaves much of it is converted into compounds that are not very mobile. These less mobile compounds probably represent the result of reactions with organic constituents in the leaves.

We should expect the mobility of boron compounds or the equilibria between mobile and non-mobile compounds as they exist in the leaves or elsewhere in the plant to affect not only the distribution of boron in the various parts of the plants, but also the rate of uptake from the soil or culture solution. Boron moved to the absorbing roots, as by transport through the phloem, would be expected to depress absorption rates; whereas very low concentrations in the absorbing root cells should tend to favor increased absorption rates.

It is reasonable to assume that mobility (4) is a factor that may have a marked effect upon absorption rates, but absorption rates as they relate to concentration potentials across absorbing membranes must likewise be considered in the light of membrane characteristics. We cannot assume that the absorbing root cells of all plants tend to maintain like concentration potentials. The data submitted in this paper show that the amount of boron accumulated by plants can be altered by substituting rootstocks. While no record has been found in the literature of an effect of rootstock on the accumulation of any electrolyte in the above-ground portions of plants, consideration has nevertheless been given to this possibility. SWINGLE, ROBINSON, and MAY (9), having observed in greenhouse tests in Washington in 1928 that *Severinia buxifolia* (a citrus relative) was more tolerant to boron than the several citrus varieties compared with it, suggested that the plant

for this reason might provide a promising rootstock for commercial citrus in areas supplied with boron-contaminated irrigation water.

Since a grafted plant may be a combination of two varieties of the same species, of two species, or even of two genera, it is surprising that so little work dealing with the effects of rootstocks upon absorption of nutrient or toxic ions should have been conducted or recorded. Only two reports of such studies have been found. ROACH (**6**) has reported the spectroscopic examination of the wood of one of two apple stocks to have shown molybdenum to be present. But this element was not found in the wood of the one scion variety grafted to these stocks. No spectroscopic evidence was obtained that the two rootstocks were different in their absorption of other elements or that the rootstock affected the accumulation of electrolytes in the scion variety. HAAS and HALMA (**2**) have shown that differences in the solubility but not in the total amount of magnesium of the bark of citrus rootstocks resulted when field-grown trees had different scions. Different rootstocks did not give rise to differences in the solubility or quantity of magnesium in the bark of the scion. The concentration or solubility of calcium was not affected either in the stock or in the scion by the several combinations studied.

The primary purpose of the studies here reported was to determine whether boron accumulation is primarily conditioned on root characteristics or on characteristics of or reactions in the above-ground organs. In electing to use reciprocally grafted plants as a method of experimental approach to this question, it was recognized as essential that the plants employed should be well contrasted with respect to boron uptake. The Jerusalem artichoke and the sunflower were selected as one plant pair and the lemon and Chinese box orange as another.

The Jerusalem artichoke (*Helianthus tuberosus*) is sensitive to boron, being severely injured when boron is present in culture solutions in concentrations as low as 5 p.p.m. The sunflower (*Helianthus annuus*), on the other hand, is only moderately tolerant to boron; its growth is not greatly reduced by culture solutions containing as much as 15 p.p.m. The artichoke, when grown beside the sunflower in the same culture solution, accumulates in its leaves higher concentrations of boron than does the sunflower. The two plants, which may be readily intergrafted, are different in an important physiological respect; inulin is the storage carbohydrate of the artichoke, whereas starch is the storage form in the sunflower.

The lemon (*Citrus limonia* Osbeck) is highly sensitive to boron; in fact few plants are thought to be more sensitive, and it accumulates much boron in its leaves. The Chinese box orange, *Severinia buxifolia,* on the other hand, in confirmation of the observation of SWINGLE, ROBINSON, and MAY, is relatively tolerant to boron. *Severinia* has been found to accumulate

much less boron in its foliage than does the lemon. The writers are indebted to SWINGLE, ROBINSON, and MAY for supplying the *Severinia* and reciprocally grafted lemon and *Severinia* plants used in these experiments.

Experimental methods

SUNFLOWERS AND ARTICHOKES

The sunflowers and artichokes on their own roots and the reciprocally grafted sunflowers and artichokes were grown out of doors in large sand culture vessels during the summers of 1932 and 1933 at Riverside, California. The base culture solution had the following composition: $Ca(NO_3)_2$, $MgSO_4$, KH_2PO_4, in concentrations of 4, 2, and 2 millimoles per liter respectively. In addition there were added $MnCl_2$ to give 0.5 p.p.m. of manganese, 1 cc. per liter of a 5 per cent. solution of iron tartrate, and boric acid in sufficient amounts to give the concentrations of boron subsequently indicated. This solution was applied daily in sufficient excess to produce 50 per cent. or more of drainage from the sand culture vessels.

In both years the plants were all grown together in a single vessel, thus assuring that they were subjected to like concentrations of the boron supplied.

In 1932 the seeds and tubers were planted on April 12 and the artichokes and sunflowers reciprocally grafted by the approach method on June 10. From time of planting until July 8 the nutrient solution contained 1 p.p.m. of boron. The same nutrient solution but with 5 p.p.m. of boron was used from July 8 until the plants were harvested on August 19. In other words, these plants received the high boron solution for 42 days at the end of which period some of the older artichoke leaves had been killed.

In 1933 the sunflower seeds were planted on April 11 and the artichoke tubers on May 14. The grafting was done during the last week of June. These plants were grown with 1 p.p.m. of boron in the nutrient solution until September 1 and thereafter until harvested on October 1 with a 5 p.p.m. solution. By September 1 all branches of both sunflowers and artichokes were terminated by a flower or flower bud; no new leaves were formed therefore by the 1933 plants during the high boron treatment. In 1932 new leaves developed after the high boron treatment had been started.

In 1932 separate samples were taken of old and young leaves but not all leaves on any plant were included. In 1933 each leaf sample included all of the leaves, either sunflower or artichoke, on a particular plant. Enough boron may accumulate in a mature Jerusalem artichoke leaf of a plant growing in a 1 p.p.m. boron solution to cause some injury, and while some boron will likewise accumulate in sunflower leaves there will be no visible evidence of injury.

In 1932 the stem samples were of 9-inch segments above and below the point of the graft on the grafted plants and above and below a comparable point on the ungrafted plants. The 1933 stem samples were of entire stems divided only as to artichoke or sunflower.

Lemons and *Severinia*

The base nutrient solution used for the lemon and orange experiments was the same as that used for the artichoke and sunflower experiments but the boron concentrations of the solutions as applied were 2, 4, and 6 p.p.m. These concentrations were used for 22 months before the samples of mature leaves were collected for analysis, February 2, 1934.

The lemons on their own roots were three years old from cuttings. The oranges on their own roots and the reciprocally grafted lemons and oranges were 3 and 4 years old and were also from cuttings.

For the most part the different plants were grown in separate 20-gallon sand culture cans, although some were paired in the same cans (table II). Except when the plants were in pairs in the same containers, it is not implied that the average boron concentrations as presented to the roots were identical for all plants of a series. Under Riverside climatic conditions the concentrations of boron in culture solutions such as were here used increase as the volumes are decreased by transpiration. The transpiration losses from the different sand-culture cans were different owing to differences in plant sizes. The 2, 4, and 6 p.p.m. boron concentrations therefore should be looked upon as minimum concentrations. The culture solutions were applied daily in quantities roughly in proportion to plant sizes and always in sufficient amount to produce copious drainage. Only mature leaves were selected for analysis. The average age of leaves making up each of the samples undoubtedly varied a little. This variation, like the possible variation in the concentration of boron in the culture solutions held by the sand in cans receiving like treatments, would affect to some extent the quantity of boron accumulated.

Experimental results

Sunflowers and artichokes

The results of the 1932 experiment are reported in table I. The concentrations of boron found in the samples of the sunflower leaves were 50 per cent. greater when this plant was grown on artichoke roots than when grown on its own roots; for the older leaves the values were 1470 and 936 p.p.m. respectively. Conversely, when the artichoke was grown on its own roots the older leaves were found to contain 1520 p.p.m. of boron whereas when on sunflower roots the older artichoke leaves contained but 711 p.p.m. of boron.

TABLE I

BORON IN LEAVES AND STEMS OF SUNFLOWERS AND ARTICHOKES WHEN ON THEIR OWN ROOTS AND WHEN RECIPROCALLY GRAFTED; 1932 EXPERIMENT

PLANT	BORON (DRY WEIGHT)
	p.p.m.
Sunflower on own roots	
Older leaves	936
Younger leaves	494
Stems: upper segment	30
Stems: lower segment	24
Artichoke on own roots	
Older leaves	1520
Younger leaves	1224
Stems: upper segment	63
Stems: lower segment	50
Sunflower on artichoke roots	
Older leaves	1470
Younger leaves	1128
Stems: sunflower segment	40
Stems: artichoke segment	43
Artichoke on sunflower roots	
Older leaves	711
Younger leaves	414
Stems: artichoke segment	21
Stems: sunflower segment	26

The sunflower leaves showed only mild injury when the plant was on its own roots but the injury was marked when on the artichoke roots (fig. 1). The artichoke leaves were severely injured when the plant was on its own roots; while on sunflower roots the injury was perhaps 60 per cent. as severe.

The results of the 1933 trials are shown diagrammatically in figure 2. The methods were different from those of 1932 in that the plants were not subjected to the high boron concentration until they had flowered, and then for only a 30-day period during which no new leaves were formed. Also in 1933 branches of the rootstock variety were allowed to develop below the grafts. All leaves of a species on each individual plant were combined as single samples irrespective of age.

As shown in figure 2, the concentrations of boron in the leaves of two sunflower plants on their own roots were 575 and 684 p.p.m., whereas sunflower leaves on plants with artichoke roots contained 1026 and 954 p.p.m. of boron. Conversely, the leaves of artichoke plants on their own roots contained 893 and 879 p.p.m. of boron, whereas on sunflower roots

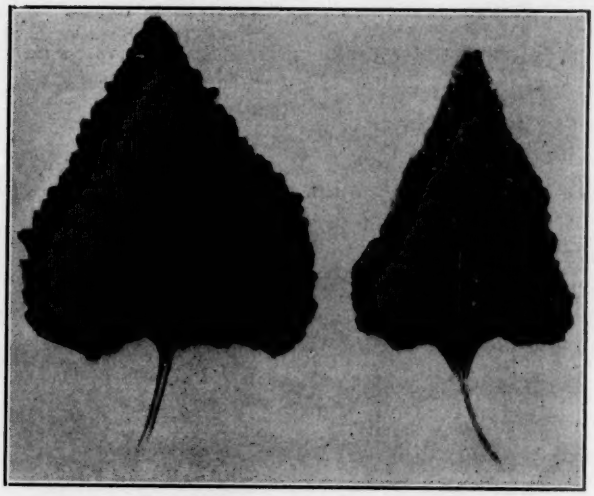


FIG. 1. Sunflower leaves of like ages: leaf on left is from a plant on its own roots and shows only a little boron injury along its margin; leaf on right is from a plant on artichoke roots and is severely injured.

artichoke leaves contained 663, 690, and 644 p.p.m. of boron. The anomaly presented, for example, by the high accumulation of boron in basal sunflower branches of plants with artichoke tops will be dealt with in the discussion.

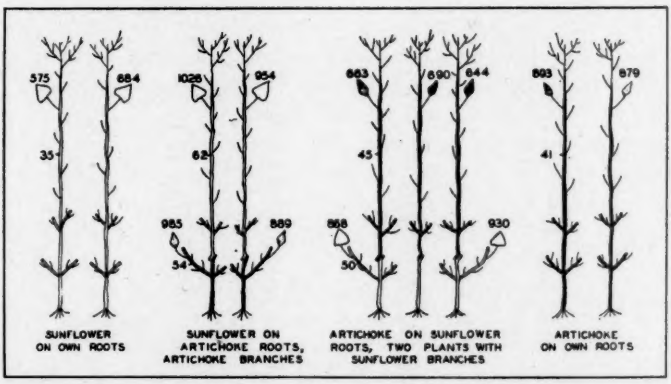


FIG. 2. Sunflowers and artichokes of 1933 experiment: boron concentrations, expressed as p.p.m. of dry weights, represent samples of all leaves and of entire stems of each plant, separated (as designated) only as to species.

LEMONS AND *SEVERINIA*

The effects of reciprocally grafting lemons and *Severinia buxifolia* on the accumulation of boron in the leaves are set forth in table II.

TABLE II

CONCENTRATIONS OF BORON IN LEMON AND *SEVERINIA* LEAVES (DRY WEIGHT) AFTER GROWING FOR 22 MONTHS IN SAND CULTURES SUPPLIED WITH NUTRIENT SOLUTION CONTAINING 2, 4, AND 6 P.P.M. OF BORON

PLANT	BORON IN CULTURE SOLUTION		
	2	4	6
	p.p.m.	*p.p.m.*	*p.p.m.*
Eureka lemon on own roots			
Can: 2B–12 4B–13	637	1065	Dead
2B–13	724		
Eureka lemon on box orange roots			
Can: 2B–10 4B–10 6B10	223	283	517
Lisbon lemon on box orange roots			
Can: 2B–13 4B–13 6B–12	253	397	457
Box orange on Eureka lemon roots			
Can: 2B–10 4B–10	528	877	.....
Box orange on own roots			
Can: 6B–11–1	.....	.....	390

These results are similar to those obtained with sunflowers and artichokes but are perhaps even more outstanding. In terms of the concentrations of boron accumulated, the lemon and box orange are more sharply contrasted than the sunflower and the artichoke.

Lemon on box orange roots had about one-third as much boron in their leaves as when grown on their own roots. The data indicate that the boron concentrations in the box orange leaves are increased threefold when the plant is grown on lemon roots. There were no box orange plants on their own roots in the 2 and 4 p.p.m. cans, but the plant in 6 p.p.m. on its own roots had 390 p.p.m. whereas the box orange on lemon roots in 4 p.p.m. had 877 p.p.m. of boron in its leaves. Lemon leaves on their own roots were more severely injured than when on the box orange roots (fig. 3).

Both Eureka and Lisbon lemons were used in these tests. We believe that the reactions of the two varieties to boron may be considered identical since no differences were found in their boron accumulation characteristics.

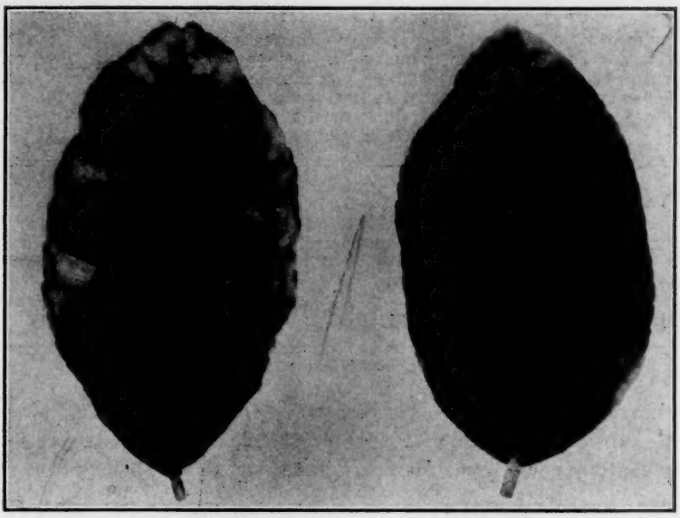


FIG. 3. Lemon leaves: leaf on left is from a plant on its own roots and shows marked boron injury; leaf on right is from a plant on *Severinia* roots and shows little boron injury.

Discussion

The data presented show that the quantities of boron accumulating within plants may be affected in an outstanding way by the character of the rootstock. The boron relations of plants, however, are known to be broader and more complex than these experiments would suggest. That the character of the rootstock does not stand alone as a factor determining boron absorption, accumulation, and injury may be illustrated by the behavior of Payne and Eureka walnuts on black walnut roots.

Under field conditions it has been found that the Payne walnut shows less severe leaf injury than the Eureka variety when the two are grown on black walnut (*Juglans hindsii* Jepson) roots in the same grove. Leaves of the Payne variety have likewise shown less injury and have uniformly contained less boron than leaves of the Eureka variety when the two were grown on black walnut roots side by side in sand cultures (table III).

Tolerance to boron is only in part a matter of non-accumulation. Marked differences exist in the concentrations of boron found in the leaves of different species showing a similar severity of injury. The Navel orange accumulates perhaps slightly more boron in its leaves than does the lemon when both are grown on like rootstocks under comparable conditions, and yet the injury shown by lemons greatly exceeds that shown by oranges. Oranges

TABLE III

BORON IN LEAVES (DRY WEIGHT) OF PAYNE AND EUREKA WALNUTS ON BLACK WALNUT ROOTS WHEN GROWN IN PAIRS IN SAME CULTURES. CULTURE "O" CONTAINED A TRACE OF BORON FROM IMPURITIES OF THE CHEMICALS AND SAND. CONCENTRATIONS OF BORON IN OTHER BEDS WERE MAINTAINED AS INDICATED. LEAVES WERE COLLECTED AT END OF FIRST GROWTH SEASON AFTER TRANSPLANTING TREES WITH YEAR-OLD BUDS FROM FIELD

VARIETY	BORON IN CULTURE SOLUTION				
	"O"	1	3	6	9
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Payne	46	162	468	728	1280
Eureka	81	260	579	902	0*

* No leaves retained; all were severely injured and abscissed.

will withstand possibly twice as much boron in the soil or culture solution before injury comparable with that of the lemon is shown. It is most reasonable to suppose that differences in the severity of injury that results from like concentrations of boron in the leaves of different plants are related, at least in part, to the nature of the boron compounds present. Some of these compounds may have a far greater toxicity than others. The first evidence of injury is manifest by a reduction in the amount of chlorophyll in the leaf; *i.e.*, portions of the leaves turn yellow. This reduction is associated with boron accumulation but as yet we are unable to conclude whether the effect is a direct or an indirect one.

Boron is known to be reactive with a number of organic compounds normally occurring in plants and to form with them complex molecules. Boric acid combines readily with ethyl alcohol to form tri-ethyl borate, $(C_2H_5)_3BO_3$, and with methyl alcohol to form tri-methyl borate, $(CH_3)_3BO_3$. Boron also unites with various higher alcohols (glycerol, erythritol, mannitol, sorbitol, dulcitol) and with certain of the sugars to form compounds of uncertain character but probably with ester-like linkages. Analytical use is made of the reaction with mannitol which, when added to a solution of boric acid, liberates one H ion for each molecule of boric acid present. SHKOL'NIK (**8**) cites data by INOZEMCEV showing that boron accelerates the gelatinization of gelatin, increases the viscosity and decreases the distention. He submits his own data to show that boron exerts an effect on the permeability of roots. Without boron in culture solutions the absorption of PO_4, NO_3, and Ca by flax and *Vicia faba* was greatly increased over control plants supplied with boron, whereas K absorption was depressed. These relationships indicate that boron has a marked effect on the permeability of the plant protoplasm and that it may combine with proteins or their derivatives, but the reactions between boron and the amino acids or proteins apparently have not been investigated.

Only a little is known about the character of organic compounds of boron, and while there may be many of them, they have received scant attention. Attempts to isolate, identify, or measure the quantities of such compounds in plant material or to trace their movement would be of uncertain outcome and scarcely warranted at the present time. The total-boron analyses presented in the introduction provide evidence that boron, after reaching the leaves, forms compounds that are not readily translocated. The same data indicate that in different plants there are differences in the extent of movement. These facts favor the complex organic-molecule interpretation of low mobility.

In the sunflower-artichoke experiments it was found that the leaves of basal sunflower branches of plants with sunflower roots and artichoke tops contained 868 and 930 p.p.m. of boron whereas sunflower leaves on plants without the artichoke top contained 575 and 684 p.p.m. of boron. These observations can be explained if we assume either that the boron of the artichoke is more mobile than that of the sunflower or that the ratio of mobile boron to non-mobile boron is higher in the artichoke than in the sunflower. On either basis a movement of boron from artichoke leaves to sunflower leaves should occur with a resultant accumulation in the sunflower leaves of boron converted into a less mobile form.

It has been suggested that differenees in transpiration rates might account for the marked variability in the boron absorption characteristics of different plants. Without attempting to minimize the possible importance of this factor, it is nevertheless desirable to point out that the total boron taken up by plants when considered with reference to the total transpiration shows the ratios of water absorbed to boron absorbed to be high. Transpiration, for example, would fail to account for the threefold effect on boron accumulation that resulted when the lemon was grown on *Severinia* roots or when *Severinia* was grown on lemon roots.

On the basis of the foregoing, two theories may be advanced as to why plants show differences in the quantity of boron absorbed. The first recognizes, as between plants, differences in the characteristics of the protoplasts of the absorbing roots that affect the equilibria between external and internal boron concentrations and therefore the rate of boron uptake. The second recognizes, as between plants, differences in the forms and in the equilibria concentrations of the non-mobile and mobile boron of the leaves and other organs that would be expected in turn to affect boron concentrations in the root cells and accordingly the rate of uptake. Low concentrations of mobile boron by the latter theory would favor low concentrations in the root cells and increased rates of uptake and relatively high concentrations of total boron in the leaves. The many facts which have been accumulated with regard to plant reactions to boron can best be explained by assuming that

both these theories are essentially valid and that both may be operative in one and the same plant at the same time, acting either in the same or in opposite directions to varying degrees.

The researches of OSTERHOUT (5) and others demonstrate that single cells may show outstanding differences in their intake equilibria. But as HOAGLAND and DAVIS (3) emphasize in their study of the intake of electrolytes by *Nitella*, the mineral nutrition of plants involves much more than a question of cell permeability. That higher plants grown on the same substratum differ in the quantity and proportion of ions they absorb needs no confirmation, for differences are the rule rather than the exception. The work of MASON and MASKELL (4) on the transport of calcium, phosphorus, nitrogen, and potassium through the phloem can leave little doubt regarding either the downward movement of several of these constituents from the leaves to the roots or their reappearance in the transpiration stream. Elements returned to the root cells through the phloem, as they believe to be the case, must almost unquestionably affect absorption rates. MASON and MASKELL'S deductions as to the mobility of various forms of nitrogen and carbohydrates conform with the conditions here deduced with respect to the mobility of boron.

The obvious conclusion to be drawn from the boron experiments is that both root-cell permeability and the mobility of the boron compounds occurring in plants affect uptake and accumulation. Whether or not valuable field application can be made of these possibilities is a question that cannot now be answered. The selection of rootstocks possessing desirable absorption characteristics can constitute only a part of inquiries directed toward practical ends, since rootstocks are to be chosen also on the basis of such factors as disease resistance, vigor, and compatibility.

The foregoing discussion naturally leads to the more general question of whether the rootstock effect here observed is limited to boron. It is recognized that the reaction of plants to high concentrations of boron is in some respects outstanding and in a measure differentiated from plant reactions to other inorganic constituents of the soil. Nevertheless there seem to be no grounds for assuming that the rootstock effect may not be a common one. A question is likewise presented as to whether certain rootstocks might be more profitably used than others for the culture of orchard crops on soils deficient in one or more nutritive elements. If the effect with respect to certain other ions is a common one, then it is not at all unlikely that a measure of advantage has already been taken of it in the choice of rootstocks in older orchard sections, even though the selections may have been made on the basis of observed suitability by a trial and error process rather than through any recognition of cause.

For the purpose of determining whether sunflowers and artichokes or Chinese box oranges and lemons were different with respect to the accumulation of calcium, magnesium, sodium, potassium, or sulphate, analyses were made of the leaves of these plants as grown on their own roots. These analyses did not show differences sufficiently well marked with respect to any of the determined ions to offer promise of significant differences in the reciprocally grafted plants.

Summary

1. There is an outstanding variability in the extent to which different plants take up boron. The boron accumulated by plants is not uniformly distributed in the different organs or parts but tends to accumulate in some parts to a much greater extent than in others. Within particular plant parts the accumulation of boron is by no means uniform; 30 times as high a concentration of boron has been found in the margins of lemon leaves as in the midveins and petioles. It is believed that boron is carried to the leaves by the transpiration stream as an inorganic radical but that on reaching the leaves much of it combines with organic compounds that are not very mobile.

2. The boron concentrations accumulating in the leaves of the scion were directly influenced by the rootstocks upon which they were grown. The scion leaf concentrations were reduced if grafted to varieties normally accumulating lesser concentrations and increased if grafted to varieties normally accumulating higher concentrations. Concentrations of boron in scion leaves tended to approach those normal to the rootstock variety. Reciprocally grafted sunflowers and Jerusalem artichokes were used in one series of experiments and lemons and Chinese box oranges in another.

3. Boron absorption is not wholly contingent upon the rootstock employed. Eureka walnuts accumulate more boron in their leaves than the Payne variety when both are grown in the same cultures on black walnut roots. Different species accumulating comparable concentrations of boron in their leaves may show marked differences in severity of injury.

4. With other conditions equal, the rate of boron absorption is determined by (1) the characteristics of the absorbing root cells, and (2) the nature of the boron compounds in a plant and the equilibria between mobile and non-mobile forms. The mobility of boron in the plant or the proportions of mobile and non-mobile boron would be expected to affect not only the distribution of boron in different parts of the plant but also the concentration of boron in the root cells and accordingly the rate of uptake.

UNITED STATES DEPARTMENT OF AGRICULTURE
DIVISION OF WESTERN IRRIGATION AGRICULTURE
RUBIDOUX LABORATORY, RIVERSIDE, CALIFORNIA

LITERATURE CITED

1. Eaton, F. M. Boron in soils and irrigation waters and its effect upon plants, with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bull. 448. 1934.
2. Haas, A. R. C., and Halma, F. F. Chemical relationship between scion and stock in citrus Plant Physiol. **4**: 113–121. 1929.
3. Hoagland, D. R., and Davis, A. R. The intake and accumulation of electrolytes by plant cells. Protoplasma **6**: 610–626. 1929.
4. Mason, T. G., and Maskell, E. S. Further studies on transport in the cotton plant. I. Preliminary observations on the transport of phosphorus, potassium, and calcium. Ann. Bot. **45**: 125–173. 1931.
5. Osterhout, W. J. V. Physiological studies of single plant cells. Biol. Reviews **6**: 369–411. 1931.
6. Roach, W. A. The chemistry of the rootstock-scion effect. I. The elements absorbed from the soil. Annual Report, 1928, 1929, and 1930. East Malling Research Station; II, Supplement. 101–104. 1931.
7. Scofield, C. S., and Wilcox, L. V. The boron content of oranges. Science n. s. **71**: 542–543. 1930.
8. Shkol'nik, M. K. Fiziologischeskio roli bora (On the physiological rôle of boron) Dokl. Akad. Nauk U. S. S. R. (Compt. Rend. Acad. Sci. U. S. S. R.) n. s. **1**: 141–146. 1934.
9. Swingle, W. T., Robinson, T. R., and May, E., Jr. Experiments on boron tolerance of citrus plants and their wild relatives. Abstract Amer. Jour. Bot. **15**: 616–617. 1928.

SALT TOLERANCE OF *RUPPIA MARITIMA* IN LAKES OF HIGH MAGNESIUM SULPHATE CONTENT[1]

ELIZABETH MCKAY

Introduction

The ability of halophytes to develop under conditions which would mean death to most vegetation is well known. Salt marsh plants, growing where sodium chloride is the chief constituent of the soil solution and where salt concentrations usually vary from 1 to 6 per cent., are the most common halophytes. Much less numerous than the salt marshes are the lakes whose chief constituent is magnesium sulphate, a compound toxic to many plants in any but very dilute concentrations. Although the plants of the two types of lakes are to some extent the same, very little work has been done on those of the magnesium sulphate lakes. In view of the common toxicity of magnesium salts to plants and the scarcity of information concerning the physiological relations of halophytes, it has seemed worth while to investigate the vegetation of certain lakes lying in north central Washington and adjacent British Columbia which are unusual in having a very high proportion of magnesium sulphate in the salt content and in supporting seed plants at salt concentrations as high as 25 or 26 per cent. Studies were made on three lakes lying in the eastern foothills of the Cascade Mountains in the northern part of Okanogan County, Washington, and British Columbia, in one of which *Ruppia maritima* L. grows abundantly, completely submerged in the salty water of the lake.

The first of these, known locally as Epsom Lake and designated in this paper as no. 1, lies about 4 miles northwest of the town of Oroville, in northern Washington, about midway between Lake Osoyoos and the Similkameen River. The geology of the region was described by JENKINS (**10**). ST. JOHN and COURTNEY (**35**) listed the phanerogams growing in the lake and on the walls of the surrounding basin. They report the banks to be of practically pure salt, in which the plants were apparently rooted. Analyses of salts taken from the surface of the water gave a content of 99.64 per cent. of $MgSO_4$.

The lake lies at an elevation of about 2000 feet, with walls of the basin reaching several hundred feet higher. The lake itself, which drains an area of about one square mile, is, when full, approximately four acres in extent and 30 feet deep. There is no outlet and drainage waters remain until evaporated during the dry summer season, when the lake is reduced to a series of small

[1] Contribution no. 39 from the Botany Department of the State College of Washington.

pools and the bed becomes white and crystalline in appearance. The surrounding vegetation is sparse.

The second lake studied, designated no. 2, lies several miles north of no. 1, in British Columbia, on a ridge of Kruger Mountain, about 8 miles from the town of Osoyoos. It is known locally as Spotted Lake, owing probably to its spotted appearance when summer evaporation has reduced it to a series of round pools. Like no. 1, Spotted Lake is the drainage basin for the surrounding hills and has no outlet. It is considerably larger than no. 1, being about one-half mile long and 300 yards wide. The elevation is somewhat greater than that of lake no. 1 but the walls of the basin are not so steep. Vegetation is even more sparse than around lake no. 1 and no sign of plant life is found in the waters of the lake except a few algae. The lake contracts to a white bed with round pools of saturated water after a dry period (**10**).

Lake no. 3 lies a few miles to the south of no. 2 and at a greater elevation on Kruger Mountain. It is much larger and considerably deeper than the others. Although it is much less concentrated than the others there is no evidence of plant or animal life and no growth of plants for some distance from the water's edge. The soil of the banks is colored and sandy, and is not so white and crystalline in appearance as that of the other two lakes.

Literature review

Until recently work on halophytes has been concerned chiefly with their ecological relations. SCHIMPER (**34**) described halophytes as those plants which are capable, owing to xeromorphic adaptations of physiological and anatomical characters, of living under conditions of physiological dryness where water, although present, is not readily available to the plant. FITTING (**7**) found halophytes able to develop osmotic pressures of sufficient value to overcome the resistance to absorption of greatly concentrated soil solutions. DELF (**4**) found the transpiration rates of *Salicornia annua* and *Suaeda maritima* comparable to mesophytes such as *Vicia faba,* the halophytes having no stomatal modifications to reduce transpiration. YAPP (**40**) described the xeromorphic character of *Spiraea ulmaria,* showing the development of characters usual in xerophytes. Apparently halophytism may be expressed in either physiological or morphological adaptations or both.

OSTERHOUT (**15**), in working with marine plants, found that each of the salts of sea water is poisonous when alone but that the mixture, as found in sea water, gives a "physiologically balanced" solution which the plant is capable of using. The idea of balance of solution was soon extended to include fresh water and terrestrial plants (**16**). Since then extensive investigations by OSTERHOUT (**17, 18, 19, 20, 21, 22, 23, 24, 25**), TRUE and

BARTLETT (**37, 38**), RABER (**27, 28, 29, 30, 31**), SCARTH (**33**), and many others have laid the basis for the theory of the antagonism of ions, have secured much information concerning the antagonistic relations of various ions, and have added greatly to our knowledge of the effects of acids, alkalies, and anions and kations of different valences on the permeability of plant membranes. OSTERHOUT (**26**) and HOAGLAND and DAVIS (**9**) have given analyses of the cell sap of *Valonia* and *Nitella* respectively, as compared with the water in which they were growing.

Recently consideration has been given to the salt requirements of salt marsh plants. Analyses made by ZELLNER (**41**) of *Salicornia herbacea, Suaeda salsa, Scorzonera parviflora, Plantago maritima, Aster tripolium,* and *Erythraea tinariafolia* growing on soils containing about 3 per cent. of soluble salts showed all of the plants to contain large amounts of Na, Cl, and SO_4. Over 20 per cent. of the dry weight was found to be ash in *S. herbacea* and *S. salsa.* These results are in accord with those of KILLIAN (**11**), who concluded, after studying plants growing in soils rich in salt, that NaCl may be in even greater concentration in the plants than in the soil, and that different species growing in the same soil may show great variation in the salt accumulated in the plant. MONTFORT and BRANDRUP (**12, 13, 14**) concluded that the resistance of plants to high salt content varies at different stages of development of the plant as well as with different concentrations of salt. The pH of the soil solution plays a minor rôle, but the proportion of Cl and SO_4 is important in determining plant distribution. RIKHTER (**32**) likewise found balance important, in that plants can tolerate much greater concentrations of balanced solutions than of pure salts. He groups plants able to tolerate high content of salt into two types with regard to the method of developing the internal osmotic pressures necessary to overcome the external pressures. Such plants as *Helianthus, Triticum, Amaranthus,* and *Artemisia* increase their own supply of osmotically active materials, while halophytes such as *Salsola, Suaeda,* and *Salicornia* can accumulate salts from the outside medium without harm to themselves. Plants of the first group are characterized by a high impermeability of the roots, so that the power to exclude the salts determines the limit of concentration which the plants can endure. These workers were interested in plants growing in salt concentrations close to that of sea water. BRIGHENTI (**2**) describes salt marshes of the province of Ferrara, Italy, near the Adriatic Sea. These have a depth of 50–90 cm. In summer the NaCl reaches 41 per cent. concentration, although this is diluted in the winter by rain and by the Po di Volano. No phanerogams grow in the marshes but some diatoms are found, and the algae *Ulva latissima* and *Chaetomorpha crassa* are abundant.

FLOWERS (**8**) describes the vegetation of the Great Salt Lake region, listing the plant associations of the lake, the dunes, salt marshes, etc., and giving ecological data including the salt content of soils and soil waters. He compiles tables of analyses made of Great Salt Lake water at different periods from 1877 to 1910. The salt content varied from 13.79 to 27.72 per cent. during the period. NaCl is the principal constituent. The Cl analyses of the total salt vary from 55.1 to 56.54 per cent. Na varies from 32.81 to 34.65 per cent.; SO_4 from 5.95 to 6.82 per cent.; and Mg from 0.57 to 3.18 per cent. The main body of the lake supports no vegetation except blue-green algae, of which *Apanothece utahensis* is at present dominant. Three species of animals are listed by FLOWERS, of which one is *Artemia gracilis*. *Ruppia maritima* is reported as growing in Willard Spur, a part of Bear River Bay, where the strong brines of the main body of the lake are considerably diluted.

While magnesium in minute amounts is essential for all plants, to many it is toxic in very slightly increased amounts. Considerable variation is shown by different plants. EILERS (**5**), investigating the physiology of nutrition of the alga *Stichococcus bacillaris*, found that this was susceptible to relatively small amounts of magnesium salts. CARMIN (**3**) grew very young wheat seedlings in solutions of 0.0005 to 0.0150 M solutions of $MgSO_4$. When growth of the roots and tops was plotted against the concentration of $MgSO_4$, the curves showed lessening of the growth with greater concentration. The salt was more toxic to the roots than to the tops. TRELEASE and TRELEASE (**36**) detected injury due to magnesium toxicity in seedlings of wheat, oats, rye, and barley. The severity of injury increased as the Mg/Ca ratio increased from 1 to 50, showing the injury to be the result of disturbance of balance among the ions.

Very different results were obtained by ESPINO and PALISOC (**6**), who found that young rice plants grow well in a culture solution containing one part KH_2PO_4, one part $(NH_4)_2SO_4$, one part $Ca(NO_3)_2$, and thirty-two parts $MgSO_4$ with a total concentration of 0.00875 mols of salt per liter. $MgSO_4$ was necessary, apparently, as $Mg(NO_3)_2$ and $MgHPO_4$ produced plants with dry and chlorotic tips on the leaves.

WORONICHIN (**39**) reports a preliminary investigation of the relation of $MgSO_4$ deposit on the bottoms of lakes near Pjatigorsk to the plant growth in the lake. Three periods of development are described. In the first period, one of low and medium amounts of salt content, there is a growth of *Ruppia*. In the second period there is a decline in the *Ruppia* and vegetative growth of *Vaucheria dichotoma*. In the third period there is a rapid salting, decline of *Vaucheria* and the building of felt. Several new Myxophyceae are described: *Synechocystis minuscula, Oscillatoria*

tambi, and *Oscillatoria kützingiana* var. *crassa.* A green alga, *Carteria flos aquae,* is also listed.

Collection of material

Collecting trips were made to lake no. 1 at three different times. The first was in May of 1931, after several weeks of dry weather. The lake was reduced to a series of pools in which the water was greatly concentrated. The banks were brilliantly white, appearing to be practically pure salt deposits, although digging below the surface showed the mud underneath to be black in appearance and very ill-smelling. At the time of the first visit there was an abundant growth of seed-bearing *Ruppia maritima* and blue-green and green algae in the water. Great numbers of a small red crustacean, the "brine shrimp," *Artemia salina,* were swimming in the water. No plant collections were made, but several liters of water were taken and some of the mud was collected. When dried this was white and crystalline, being nearly identical in appearance with the salts obtained by evaporation of the water. It is in this mud, with its dry, white top layer, that the plants root. As the season advances, progressively deeper layers dry and become white, finally leaving the root system of *Salicornia* and other plants in what appears to be pure salt.

The second trip was on May 19, 1933. The season had been much rainier than that of two years earlier, and a fairly heavy rain had fallen the day before the trip was made. The pools of the lake were connected, the salt concentration in the water was much less than at the previous visit, and the banks, instead of being brilliant and white, were dull gray in appearance, giving evidence of their being composed of more than pure salt. *Ruppia maritima* was growing in even greater abundance than previously and was flowering, but there was little algal growth except in a small pool at one side, where the concentration was greater. *Artemia salina* was abundant in the lake, and a small red Cyclops was also present. Collections were made both of *Ruppia* and of the water in which it was growing.

The third collection was made July 22, 1933. Again the lake had evaporated to a series of pools, the banks around were white, and the water concentrated, although not so greatly as at the visit in 1931. *Ruppia* was growing somewhat less luxuriantly than in May and with a greater admixture of blue-green and green algae. The *Ruppia* had developed seeds about 1–2 mm. in diameter, black and shiny in appearance. Collections were made of the plant material and of the water.

Lake no. 2 was visited in May but no collections were made. The lake was continuous at that time, with grayish white banks. Occasional piles of salt on the bank were snowy in appearance. Except for a few algae, there was no sign of plant life in the waters of the lake.

The lake was visited again in July, 1933. The bed was an expanse of white salt with numerous round pools of saturated water, many of which were nearly covered with layers of salt which had crystallized out on top of the water. No phanerogams were found in the lake, but small numbers of a minute, unicellular, motile, yellowish green alga were seen, probably accounting for the yellowish green appearance of the pools from the hill above. Collections were made of the water and the alga, but the alga was not determined.

On the July trip to lakes no. 1 and no. 2 a stop was made at lake no. 3 and water was collected.

All three of the lakes are highly poisonous to the cattle which graze in the region and are tightly fenced to protect stock from access to them. Several carcasses lying within a few hundred feet of the lakes were testimony to the rapidity with which death followed when an animal did drink the water.

That the accumulation of salts is due to the lack of outlets usual to lakes is evidenced by the fact that some of the lakes lying between no. 2 and no. 3 were supporting an abundant growth of plants usual to lake waters and were left accessible to the stock in the region.

Material and methods

Data were obtained on the water and plant material from the three collections of May, 1931, May, 1933, and July, 1933. This included five samples of water, three from lake no. 1, one from lake no. 2, and one from lake no. 3. The plant collections included those of May, 1933, from lake no. 1 and those of July from lake no. 1. The specific gravity of the water was measured at 26° C. The pH was determined by means of a quinhydrone electrode. Concentration of dissolved substances was obtained by evaporation and drying at 100° C. The osmotic pressure was determined by measurement of the freezing point with a Beckmann thermometer. Ash analyses were made, using the methods of the Official Association of Agricultural Chemists, for Mg, Na, K, Ca, Fe and Al, SO_4, Cl, PO_4, and NO_3. No analyses were made for sulphide or sulphite, although traces of sulphide were undoubtedly present.

The plant material was washed thoroughly with tap water, dried at 63° C., and the moisture content determined.

The soluble portion of the plant was extracted by boiling and expressing the sap by pressure. The sample was weighed, distilled water was added and boiled, and the sap was pressed out. Then the whole extract was evaporated to the volume of the original sap as determined by weight of the original sample and weight of the insoluble material. Measurements were then made of the specific gravity, pH, and osmotic pressure of the sap.

Determinations were made for soluble sugars in the sap by the Shaffer-Hartmann method and for protein N by the Kjeldahl method. Ash analyses were made on both the sap and the insoluble residue for Mg, Fe and Al, Na, K, Ca, Cl, SO_4, PO_4, and NO_3.

As it was thought possible that arsenic might be found in some of the lakes, qualitative analyses were made for it using Marsh's test. No traces were found in any of the five samples analyzed.

In addition to these data, some observations were made on the growth of *Ruppia* and of *Artemia* from the May, 1933, collections in different culture solutions. The solutions were made up as follows: lake water, from the May, 1933, collection from lake no. 1; equal parts of lake water and distilled water; two-thirds lake water and one-third 2 per cent. $MgCl_2$ solution; one-third lake water and two-thirds 2 per cent. $MgCl_2$ solution; Knop's solution; and Knop's solution with additional $MgCl_2$ to bring the osmotic pressure up to that of the lake water. About a quart of each solution was placed in a 2-quart glass jar and a few plants of *Ruppia* were placed in each. Records were made of the vigor of the plants as well as this could be determined by observation.

Three specimens of *Artemia*, a small amount of algal material from lake no. 1, and some culture solution were placed in each of a group of small beakers, and observations were made of the behavior of the crustacean. The culture solutions were as follows: lake water, from the May collection of 1933; one-half lake water and one-half 4 per cent. $MgCl_2$ solution; 2 per cent. $MgCl_2$ solution; 4 per cent. $MgCl_2$ solution; 12 per cent. $MgCl_2$ solution; distilled water; and 22 per cent. solution of salts obtained from evaporation of the water collected in May, 1931.

Data

Analyses of the water samples for NH_3 showed traces in the water. Data accumulated in measurements and analyses of the samples of water and of plant material are listed in tables I to IX.

Figures obtained for the moisture content of the plants were: May collection, 92.2 per cent.; July collection, 90.8 per cent.

The cultures of *Ruppia* showed varying results. None of the plants remained alive after 45 days. Death at that time was probably due to their being placed in rather narrow-mouthed jars with no special care taken to provide for aeration. Those in Knop's solution were the first to succumb, being dead and decomposing within 15 days. Those in Knop's solution and $MgCl_2$ with an osmotic pressure equal to that of the lake water survived only a few days longer than the plants in Knop's solution alone. The plants in two-thirds lake water and one-third 2 per cent. $MgCl_2$ behaved much the same as those in one-third lake water and two-thirds 2 per cent. $MgCl_2$

TABLE I

WATER COLLECTED FROM LAKE NO. 1, MAY, 1931. APPROXIMATE CONCENTRATION, 26%; SPECIFIC GRAVITY AT 23° C., 1.21; OSMOTIC PRESSURE, 58.0387 ATM.; pH, 7.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	36.6145	1.5055	3.0110	40.1	30250
Na	8.60	0.3739	0.3739	9.96	7100
K	1.6651	0.0426	0.0426	1.13	1380
Ca	0.0912	0.0023	.0046	0.06	75
Fe and Al	Trace				
Total	46.9708	1.9243	3.4321	51.25	38805
SO_4	169.7694	1.7684	3.5368	47.16	140305
Cl	1.935	0.0546	0.0546	1.45	1600
NO_3	0.1429	0.0023	0.0023	0.06	120
PO_4	0.0131	0.0001	0.0003	.....	10
Total	171.8604	1.8254	3.5940	48.67	142035

Total molar concentration, 3.7497 mols per liter.

TABLE II

WATER COLLECTED FROM LAKE NO. 1, MAY, 1933. APPROXIMATE CONCENTRATION, 1.6%; SPECIFIC GRAVITY AT 26° C., 1.012; OSMOTIC PRESSURE, 3.797 ATM.; pH, 8.4

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	2.1207	0.0872	0.1744	38.98	2100
Na	0.6161	0.0268	0.0268	11.98	610
K	0.0981	0.0025	0.0025	1.12	97
Ca	0.0084	0.0002	0.0004	0.08	8
Fe and Al	Trace				
Total	2.8433	0.1167	0.2041	52.16	2815
SO_4	9.6902	0.1009	0.2018	45.10	9575
Cl	0.2070	0.0058	0.0058	2.59	200
NO_3	0.0142	0.0002	0.0002	0.08	15
PO_4	0.0007	.....	.....	.....	.....
Total	9.9121	0.1069	0.2078	47.77	9790

Total molar concentration, 0.2236 mols per liter.

TABLE III

WATER COLLECTED FROM LAKE NO. 1, JULY, 1933. APPROXIMATE CONCENTRATION, 6%; SPECIFIC GRAVITY AT 26° C., 1.045; OSMOTIC PRESSURE, 12.28 ATM.; PH, ABOVE 8.5

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg.	10.3757	0.4266	0.8532	39.89	9930
Na	2.3050	0.1002	0.1002	9.37	2200
K	0.4557	0.0116	0.0116	1.08	440
Ca	0.0027	0.0001	0.0002	0.01	3
Fe and Al	Trace				
Total	13.1391	0.5385	0.9652	50.35	12573
SO_4	49.4723	0.5153	1.0306	48.18	47340
Cl	0.544	0.0153	0.0153	1.43	520
NO_4	0.0196	0.0003	0.0003	0.03	20
PO_4	0.0026				2
Total	50.0385	0.5309	1.0462	49.64	47882

Total molar concentration, 1.0694 mols per liter.

TABLE IV

WATER COLLECTED FROM LAKE NO. 2, JULY, 1933. APPROXIMATE CONCENTRATION, 48%; SPECIFIC GRAVITY AT 26° C., 1.380; OSMOTIC PRESSURE,* 125.80 ATM.; PH, 7.28

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	46.565	1.9146	3.8292	24.45	33740
Na	51.5239	2.2402	2.2402	28.61	37330
K	11.3754	0.2909	0.2909	3.71	8240
Ca	0.0009				
Fe and Al					
Total	109.4652	4.4457	6.3603	56.77	79310
SO_4	314.15	3.2724	6.5448	41.79	227644
Cl	3.42	0.1123	0.1123	1.43	2480
NO_3	0.0047	0.0001	0.0001		3
PO_4	0.0247	0.0003	0.0009		18
Total	317.5994	3.3851	6.6581	43.22	230145

Total molar concentration, 7.8308 mols per liter.

* To measure the freezing point depression it was found necessary to dilute the water to one-fourth the original concentration. The pressure given is calculated on that basis and is somewhat high.

TABLE V

WATER COLLECTED FROM LAKE NO. 3, JULY, 1933. APPROXIMATE CONCENTRATION, 2.4%; SPECIFIC GRAVITY AT 26° C., 1.020; OSMOTIC PRESSURE, 6.506 ATM.; pH, ABOVE 8.5

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	2.8152	0.1158	0.2316	32.53	2760
Na	1.312	0.0570	0.0570	16.01	1290
K	0.7704	0.0197	0.0197	5.53	755
Ca	0.0124	0.0031	0.0062	0.87	12
Fe and Al	Trace				
Total	4.9100	0.1956	0.3145	54.94	4817
SO_4	14.6053	0.1503	0.3006	42.22	14320
Cl	0.3472	0.0098	0.0098	2.75	340
NO_3	0.0106	0.0002	0.0002	0.05	10
PO_4	0.0128	0.0001	0.0003	0.03	12
Total	14.9759	0.1604	0.3109	45.05	14682

Total molar concentration, 0.3560 mols per liter.

TABLE VI

PLANT SAP FROM COLLECTION OF MAY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C., 1.020; SOLUBLE SUGARS, 1.109 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE, 6.637 ATM.; pH, 6.12; SOLUBLE PROTEIN NITROGEN, 0.27%

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	1.876	0.0771	0.1542	31.40	1840
Na	0.4746	0.0206	0.0206	8.39	465
K	0.2800	0.0072	0.0072	2.93	275
Ca	0.3494	0.0087	0.0174	3.54	340
Fe and Al	Trace				
Total	2.9800	0.1136	0.1994	46.26	2920
SO_4	6.693	0.0697	0.1394	28.39	6560
Cl	1.595	0.045	0.045	18.33	1560
NO_3	0.9876	0.016	0.016	6.51	970
PO_4	0.1135	0.0012	0.0036	0.48	110
Total	9.3891	0.1319	0.2040	53.71	9200

Total molar concentration, 0.2455 mols per liter.

TABLE VII

INSOLUBLE PLANT MATERIAL FROM COLLECTION OF MAY, 1933, LAKE NO. 1

	ASH ANALYSIS	
	GRAMS PER 100 GRAMS DRIED INSOLUBLE RESIDUE	INSOLUBLE MATERIAL
	gm.	%
Mg	1.8404	1.84
Na	0.3600	0.36
K	0.2124	0.21
Ca	1.1369	1.14
Fe and Al	Trace	
Total	3.5497	3.55
SO_4	2.36	2.36
Cl	0.6588	0.66
NO_3	0.7088	0.71
PO_4	0.0011	.....
Total	3.7287	3.73

TABLE VIII

PLANT SAP FROM COLLECTION OF JULY, 1933, LAKE NO. 1. SPECIFIC GRAVITY AT 26° C., 1.025; SOLUBLE SUGARS, 1.06 MG. GLUCOSE PER CC.; OSMOTIC PRESSURE, 9.755 ATM.; SOLUBLE PROTEIN NITROGEN, 0.51%

	ASH ANALYSIS				
	GRAMS PER LITER	MOLS PER LITER	EQUIVALENTS PER LITER	TOTAL MOL CONC.	P.P.M.
	gm.			%	
Mg	2.936	0.1207	0.2414	27.48	2865
Na	1.2668	0.0551	0.0551	12.54	1235
K	0.8817	0.0225	0.0225	5.12	860
Ca	0.4237	0.0106	0.0212	2.41	415
Fe and Al	None				
Total	6.5082	0.2089	0.3402	47.55	5375
SO_4	15.120	0.1575	0.3150	35.86	14750
Cl	2.0409	0.0576	0.0576	13.11	1990
NO_3	0.5128	0.0054	0.0054	1.22	500
PO_4	0.9356	0.0098	0.0294	2.23	910
Total	18.6093	0.2303	0.4074	52.42	18150

Total molar concentration, 0.4392 mols per liter.

TABLE IX

INSOLUBLE PLANT MATERIAL FROM COLLECTION OF JULY, 1933, LAKE NO. 1

	ASH ANALYSIS	
	GRAMS PER 100 GRAMS DRIED INSOLUBLE RESIDUE	INSOLUBLE MATERIAL
	gm.	%
Mg	0.619	0.62
Na	0.8108	0.81
K	0.4783	0.48
Ca	0.6151	0.62
Fe and Al	.....	
Total	2.5232	2.53
SO_4	5.845	5.84
Cl	0.1093	0.11
NO_3	0.3544	0.35
PO_4	0.6128	0.62
Total	6.9215	6.92

solution, showing a slight browning and a lessening in oxygen given off within two days. Death was gradual, however. The brown color increased slowly and at the end of 40 days some of the plants were still green. Likewise, little difference was shown between the plants grown in normal lake water and those grown in half lake water and half distilled water. These groups survived the best, being apparently healthy at the end of 16 days. By the end of 40 days they were showing some brown color, although somewhat less than the others, and shortly after they were also dead. It is interesting to note that a heavy growth of algae took place in the jars upon death of the *Ruppia*. No attempt was made to identify these, but the jars which contained Knop's solution and no lake water showed what appeared to be primarily a Chlorophycean growth, while those with the lake water appeared to have a Myxophycean growth.

Artemia was much quicker in responding to differences of environment. Those allowed to remain in lake water were not affected, being alive and showing no evidence of sluggishness at the end of 15 days, when the observations were discontinued. Those in half lake water and half 4 per cent. $MgCl_2$ solution were less affected than most of the others. At the end of 24 hours they were somewhat sluggish but recovered from this and survived for some days. At the end of six days one had died, and in four days more all were dead. The specimens placed in 22 per cent. salt from lake no. 1 survived for four days, when two died. The remaining one lived two days longer. Distilled water was the most unfavorable medium. Two of

the specimens were dead within five hours; the remaining one was sluggish and died within another three hours. Those in 2 per cent. $MgCl_2$ solution survived slightly better but were slow in movement at the end of eight hours and dead within 24 hours. Two of the *Artemia* in 4 per cent. $MgCl_2$ solution died within five hours; the other, although not active, was alive at the end of 24 hours but died a few hours later. Those in 12 per cent. $MgCl_2$ did not succumb until nearly 48 hours had passed. Those of the organisms which had died in solutions containing no $MgCl_2$ lost the red color but remained colorless after death. Those in the solutions containing $MgCl_2$ not only lost the red pigment upon dying but turned black. The black color appeared first along the mid part of the dorsal side and gradually spread out through the rest of the crustacean.

Analysis of data and conclusions

Comparison of the samples collected from lake no. 1 (tables I, II, III) shows variation in concentration from 1.6 to 26 per cent. at different seasons. *Ruppia maritima* is able to grow well under these concentrations, although the most luxuriant vegetative growth was at the lower concentration. The fact that the *Ruppia* had already produced seeds in May, 1931, when the concentration was very high, while the plant was flowering in May, 1933, at a concentration of slightly more than 1.5 per cent., indicates that fruiting is more dependent upon concentration than upon the season. The increase in concentration from 1.5 per cent. in May to 6 per cent. in July was accompanied by the development of the seeds to maturity. The algal growth, particularly of blue-green algae, was more abundant in the solutions of greater concentration.

Comparison of the salt contents of the three collections shows only small variations in the relative proportions of ions present. The Mg content is almost constant while that of the SO_4, although somewhat higher than the Mg, is only slightly more variable. These total between 84 and 88 per cent. of the total salt analyzed. While the K content remains practically the same for the two May collections and drops slightly for the July sample, the Na and Cl are both slightly greater for the dilute waters of the May, 1933, sample. Ca, at all times scant in amount, drops in the July collection. NO_3 behaves much as Ca; and Fe, Al, and PO_4 are at all times negligible in amount.

Table IV shows the analysis of lake no. 2. Here the concentration has reached the saturation point, so that, even on the hot July day when the collection was made, crystallization of salts had partially covered the water with a layer of salt. In this lake Ca, Fe, Al, NO_3, and PO_4 are practically lacking. The amount of Cl is about the same as that in tables I and III. Although the amount of SO_4 is somewhat less than that in lake no. 1, the

greatest difference lies in the amounts of Mg, Na, and K. The Mg content is about three-fifths that of lake no. 1 while the Na is almost three times as great. And the K is slightly more than three times as great as in lake no. 1. As lake no. 2 showed no organic life except the unidentified alga, the failure of plant life to grow there, when it is abundant in lake no. 1, might be due to the extreme concentration of total salt, to the lack of the essential Ca, PO_4, NO_3, and Fe, or to the greater proportion of K and Na in the solution.

The analyses of the third lake are given in table V. The concentration (2.4 per cent.) is less than in either of the other lakes in July, although greater than lake no. 1 in May, 1933. There are traces of Fe and Al. NO_3, PO_4, and Ca especially are more abundant than in lake no. 1. SO_4 is present in nearly the same amount (42.22 per cent.) as in lake no. 2. The proportions of Mg, Na, and K are again different. There is about four-fifths as much Mg as in lake no. 1 in July, somewhat less than twice as much Na, and about five times as much K. With comparisons of the actual amounts in the water from lake no. 1 in May, 1933, and lake no. 3 (which are not greatly different in concentration), it is seen that slightly more Mg and much more Na and K are present in lake no. 3. The amounts are nevertheless much smaller in either of these samples than in the water of lake no. 1 in May, 1931, or July, 1933, when plants were growing abundantly. Comparison of the three lakes suggests that the toxicity of lakes 2 and 3 to plant life lies neither in the high concentration nor in the lack of Ca, NO_3, and PO_4, but rather in the relatively high proportion of Na and K in the total salt content.

This conclusion is supported by the distribution of plant life in Great Salt Lake, as reported by FLOWERS (8). The plant life of the lake is entirely algal, although the concentration reaches no greater value than that of the magnesium sulphate lake no. 1 described here. The chief constituent, however, is NaCl rather than $MgSO_4$. The growth of *Ruppia* in less concentrated bays of Great Salt Lake shows that concentration of NaCl is evidently the limiting factor.

Tables VI and VII show the results of analyses on the soluble sap and the insoluble residue of the plant material collected in May, 1933. Tables VIII and IX show the results of analyses of the July collection. These show a high content of both Mg and SO_4. This may be due in part to failure to remove all of the external $MgSO_4$ in washing preparatory to extracting the sap, but the content is high in the July collection as well as the May. As the former was washed even more thoroughly than the latter, the amount of salt due to external $MgSO_4$ is probably not great.

Comparison of the sap of *Ruppia* from the May collection with the July collection shows a slight decrease of soluble sugars and an increase of soluble

protein nitrogen. The total molar concentration of the salts for which analyses were made has increased from 0.2455 to 0.4392 mols per liter. Tables VI and VIII show the actual amount of salt to have increased during the period from May to July in all cases except that of NO_3. This loss of NO_3 is probably due to the increase in protein nitrogen. The relative molar concentrations have changed considerably. There is a decrease in the Mg and Ca and an increase of Na and K. Likewise the SO_4 and PO_4 have increased, the latter greatly, while the Cl and NO_3 have decreased.

Selective absorption of ions by the plant is shown by the concentrations of different ions in the plant sap and the water in which it is growing. Tables II and VI give these values for the May samples. Mg and SO_4 are high in both, showing the plant able to tolerate these internally, but the amount is greater in the water than in the sap. The water contains 2.1207 gm. of Mg and 9.6902 gm. of SO_4 per liter while the sap contains 1.876 gm. of Mg and 6.693 gm. of SO_4 per liter. The water contains 0.6161 gm. of Na per liter while the sap contains 0.4746 gm. per liter. The remainder of the ions, especially Ca, NO_3, and PO_4, are more abundant in the plant sap than in the water. While there are 0.0981 gm. per liter of K and 0.2070 gm. per liter of Cl in the water, the sap contains 0.2800 gm. of K and 1.595 gm. of Cl. The water contains 0.0084 gm. and the sap contains 0.3494 gm. of Ca per liter; the water contains 0.0142 gm. and the sap 0.9876 gm. of NO_3 per liter; the water contains 0.0007 gm. and the sap 0.1135 gm. of PO_4 per liter.

Similar results are shown by comparison of the sap of the July collection and the water (tables III, VIII), although the concentrations are greater in both sap and water than in the May material. The water contains 10.3757 gm. of Mg, 49.4723 gm. of SO_4, and 2.3050 gm. of Na per liter while the plant contains 2.936 gm. of Mg, 15.120 gm. of SO_4, and 1.2668 gm. of Na per liter. As in the May material, the other ions are more abundant in the plant than in the water. The water contains 0.4557 gm. of K, 0.0027 gm. of Ca, 0.544 gm. of Cl, 0.0196 gm. of NO_3, and 0.0026 gm. of PO_4 while the plant sap contains 0.8817 gm. of K, 0.4237 gm. of Ca, 2.0409 gm. of Cl, 0.5128 gm. of NO_3, and 0.9356 gm. of PO_4. There has been considerable change in the ratios of material in the plant to material in the water from those shown in the May material. While the ratios of K, of Cl, and of NO_3 in the sap to that in the water have decreased from May to July, the ratios of Ca and of PO_4 in the sap to that in the water have increased greatly.

The contents of both plant sap and water may be compared with those of plants growing in sea water and in pond water. OSTERHOUT (**26**) reports the concentration of *Valonia* and of the sea water in which it grows. His results, converted from parts per thousand to parts per million for purposes of comparison, are given in table X.

TABLE X

	Conc. in sea water	Conc. in cell sap
	p.p.m.	*p.p.m.*
Mg	1310	Trace
Na	10900	2100
K	500	20100
Ca	450	700
SO_4	3300	5
Cl	19600	21200

With regard to the water, the difference lies chiefly in high NaCl in the sea water and high $MgSO_4$ in the lakes. The plant sap analyses are interesting. *Valonia* excludes Mg and SO_4 almost entirely and allows little Na to enter, although the concentration is high in the water. Cl and Ca are absorbed in somewhat greater concentration, and K is many times more concentrated than in the water.

In *Ruppia* the sap shows many times as much PO_4, NO_3, Ca, and K as the water. Cl is likewise selectively absorbed by the plant and Na is excluded to some extent. Unlike *Valonia, Ruppia* is able to tolerate Mg and SO_4 in large quantities, allowing a greater amount of these than any of the other ions to enter. Hoagland and Davis (9) made analyses of *Nitella clavata* which was growing in pond water (table XI).

TABLE XI

	Weight	
	Water	Cell sap
	p.p.m.	*p.p.m.*
Mg	41	430
Na	5	230
K	Trace	2120
Ca	31	410
SO_4	31	800
Cl	32	3220
NO_3	34	0
PO_4	0.4	350

In this case the amounts of Mg, SO_4, and Cl are not greatly different from one another and approximate the amounts of Ca and NO_3 in the water. Na is small in amount. The greatest difference from the water of lake no. 1 is in the much smaller concentration of total salts. While some Mg and SO_4 have been taken up by *Nitella,* the amount is much less than that tolerated by *Ruppia,* while the amounts of K and Cl are much greater.

Determinations of the pH of the five samples of lake water show all of them to be alkaline, three of them being 8.4 or above. Apparently the pH of the lakes has little influence on plant growth, as one of the lower readings (7.4) was obtained from the May, 1931, collection from lake no. 1 where plants were abundant, and the other (7.28) from the highly concentrated water of lake no. 2 where very little life was found.

Osmotic concentration determinations show the water samples to vary from 3.797 atmospheres in lake no. 1 in May, 1933, to nearly 125 atmospheres in lake no. 2 in July, 1933. In July the osmotic pressure of lake no. 1 was 12.28 atmospheres. The osmotic concentration of lake no. 1 in May of 1931, the most concentrated sample in which *Ruppia* was growing, was 58.0387 atmospheres.

The plant sap determined in May, 1933, had an osmotic concentration equal to 6.637 atmospheres, or 2.84 atmospheres more than the water surrounding it. By July the concentration of the plant sap had increased to 9.755 atmospheres. In the meantime, however, the concentration of the water had increased until the equivalent osmotic pressure was 12.28 atmospheres, or 2.525 atmospheres above that of the plant. It was not possible to determine, from the collections made, the stage of development of plants of *Ruppia* when the osmotic pressures shifted to make the external greater than the internal pressure.

The responses of *Ruppia* and *Artemia* to different culture conditions indicate that SO_4 is necessary when Mg is present, as the substitution of Cl for SO_4 resulted in rather quick death for *Artemia* and somewhat slower death for *Ruppia*. Distilled water was quickly fatal for *Artemia;* and Knop's solution, a satisfactory culture solution for most plants, caused or allowed *Ruppia* to die within a rather short period. Apparently these organisms are growing in unusual conditions, not only because they survive where other plants and animals cannot but also because they themselves require unusual conditions for growth. It is of interest, in connection with the study of *Artemia* in different culture solutions, to review the work of BOONE and BAAS-BECKING (**1**). They report KCl as being very toxic to *Artemia salina* although the adult *Artemia* survives in solutions of $MgCl_2$, $CaCl_2$, HCl, and NaCl. Only NaCl and NaBr solutions are found to cause normal ecdysis, even the chlorides of Ca and Mg being toxic after a few days.

The range of concentrations reported as being suitable for life in *Artemia* is 0 to 3.5 mols. BOONE and BAAS-BECKING (**1**) state that increasing Mg content of the brine is probably a factor in the disappearance of *Artemia*. These conclusions agree with those arrived at by growing *Artemia* in so far as the eventual toxicity of $MgCl_2$ is concerned. However, in view of the fact that *Artemia* was growing in the water collected in May, 1931,

which had a concentration of not less than 3.75 mols, of which 40 per cent. was Mg, it seems necessary to extend the range of suitable concentrations somewhat and to look for some factor other than the increase of Mg as cause for the disappearance of *Artemia*.

The halophyte *Ruppia maritima* presents an example of a plant growing directly in water but under conditions of great "physiological dryness," as the concentration of salts in the water produces osmotic pressure of 58 atmospheres or more without causing the death of the plant. *Ruppia* is able to increase the internal osmotic pressure, by utilization of salts from the water surrounding it, to greater values than the external pressure. At some stage between the external pressures of 3.797 and 12.28 atmospheres, the internal osmotic pressure of *Ruppia* fails to increase as rapidly as the external pressure, resulting in greater external than internal pressure. Further study might show this shift of balance in external and internal pressures to coincide with maturation of the seeds and cessation of vegetative growth in *Ruppia*.

Ruppia is unusual in being able to tolerate magnesium sulphate in high concentrations. The percentage of Mg and SO_4 in the water surrounding *Ruppia* was 84 to 88 per cent. of the total salt content. The plant does not exclude either Mg or SO_4. On the contrary, both are found in the plant sap in greater proportionate amounts than any other ions for which analyses were made, although not in so great concentration, compared with other ions, as in the water.

Ruppia maritima, although able to develop successfully in lakes of high magnesium sulphate content, does not tolerate high concentration of NaCl. It does not grow in Great Salt Lake, where the concentration is comparable with that of lake no. 1 but where the chief constituent is NaCl. *Ruppia* is likewise not found in either of two lakes near lake no. 1, one of which shows very high concentration and the other low concentration but both of which have relatively more Na and K. Na is evidently much more toxic to *Ruppia* than is Mg. Cultures of *Ruppia* in $MgCl_2$ solutions indicate the necessity of SO_4 with Mg, suggesting an antagonism of SO_4 and Mg ions.

Summary

1. Collections were made from a lake supporting *Ruppia* in May, 1931, May, 1933, and July, 1933.

2. At the time of the first collection, the concentration of the lake water was about 26 per cent., the pH was 7.4, the specific gravity was 1.21 at 23° C., and the osmotic equivalent was 58.0387 atmospheres. *Ruppia* was abundant, there was a heavy growth of blue-green and green algae, and the crustacean *Artemia salina* was abundant. The banks of the lake were dry, crystalline, and white on top with black mud underlying. Collections were

made of the water and of the mud. Upon drying, this mud appeared white and crystalline like the layer above.

3. At the time of the second collection, May, 1933, after a rainy season, the concentration of the lake was about 1.6 per cent., the pH was 8.4, the specific gravity was 1.012 at 26° C., and the osmotic pressure was 3.797 atmospheres. *Ruppia* showed a heavy vegetative growth and was flowering. *Artemia* was abundant, but fewer algae were found than at other visits. The banks were gray and dull in appearance. Collections were made of water, of *Ruppia,* and of *Artemia.*

4. At the time of the third collection, July, 1933, after dry weather, the concentration was about 6 per cent., the pH was above 8.5, the specific gravity was 1.045 at 26° C., and the osmotic pressure was 12.28 atmospheres. Algae, *Artemia,* and seed-bearing *Ruppia* were abundant. The banks were white and crystalline in appearance. Collections were made of *Ruppia* and of water.

5. Collections of water were made at the other two lakes in July, 1933. The concentration of one lake was about 48 per cent., the pH was 7.28, the specific gravity was 1.380 at 26° C., and the osmotic pressure was about 125 atmospheres. The lake had been reduced by evaporation to many small round pools with salt crystallized on top, and the lake bed was white and crystalline with black mud under the surface layer. The concentration of the other lake was about 2.4 per cent., the pH was above 8.5, the specific gravity was 1.020 at 26° C., and the osmotic pressure was 6.506 atmospheres.

6. Sap from plants of the May collection showed a specific gravity of 1.020 at 26° C. and an osmotic pressure of 6.637 atmospheres; from the July collection, a specific gravity of 1.025 at 26° C. and an osmotic pressure of 9.755 atmospheres.

7. Quantitative analyses were made for Mg, Na, K, Ca, Fe and Al, SO_4, Cl, PO_4, and NO_3 in the sap and insoluble material of the plant collections and in each of the five water samples. Analyses were made of the water samples for NH_3, showing traces present, and for As, showing no trace of any present.

8. The analyses show 40 per cent. of the total molar concentration of the salt in the lake supporting *Ruppia* to be due to Mg; 45 to 48 per cent. to be due to SO_4; and 9 to 11 per cent. to be due to Na.

9. The pH is apparently not a factor in plant distribution in these lakes.

10. *Ruppia* is able to tolerate Mg and SO_4 in high concentrations internally as well as externally, evidently using these in developing its own osmotic pressure.

11. The limiting factor in the development of *Ruppia* in lakes not supporting it is evidently a greater proportion of Na_2SO_4.

12. Cultures of *Artemia* and *Ruppia* indicate that $MgCl_2$ cannot replace $MgSO_4$.

13. Time of flowering and seed development of *Ruppia* are evidently influenced by the concentration of the lake in which it grows.

14. *Ruppia* was growing in external osmotic pressures of 3.797 atmospheres with an internal pressure of 6.637 atmospheres, and 12.28 atmospheres with an internal pressure of 9.755 atmospheres.

The writer wishes to thank Dr. HARRY F. CLEMENTS for suggestion of the problem, for assistance in collection of material, and for helpful criticism throughout the course of the work.

STATE COLLEGE OF WASHINGTON
PULLMAN, WASHINGTON

LITERATURE CITED

1. BOONE, ELEANOR, and BAAS-BECKING, L. G. M. Salt effects on eggs and nauplii of *Artemia salina* L. Jour. Gen. Physiol. **14**: 753–763. 1931.
2. BRIGHENTI, DINO. Ricerche biologiche sulle valli salse della Mesola. Boll. Pesca, Piscicolt e Idrobiol. (Roma) **5**: 492–537. 1929. (Biol. Abst. **3**: 7130.)
3. CARMIN, JOSEPH. Growth and variability of wheat seedlings in magnesium sulphate solutions. Bull. Torrey Bot. Club **58**: 179–190. 1931.
4. DELF, M. Transpiration and behavior of stomata in halophytes. Ann. Bot. **25**: 485–505. 1911.
5. EILERS, H. Zur Kenntnis der Ernährungsphysiologie von *Stichococcus bacillaris* Näg. Rec. Trav. Bot. Néerland **23**: 362–395. 1926.
6. ESPINO, R. B., and PALISOC, ELEUTERIO. Tolerance of young rice plants to relatively large amounts of magnesium sulphate contained in complete culture solution. Philippine Agr. **20**: 269–286. 1931.
7. FITTING, H. Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitschr. Bot. **3**: 209–275. 1911.
8. FLOWERS, SEVILLE. Vegetation of the Great Salt Lake region. Bot. Gaz. **95**: 353–418. 1934.
9. HOAGLAND, D. R., and DAVIS, A. R. The composition of the cell sap of the plant in relation to the absorption of ions. Jour. Gen. Physiol. **5**: 629–643. 1923.
10. JENKINS, O. P. Spotted lakes of epsomite in Washington and British Columbia. Amer. Jour. Sci. **46**: 638–644. 1918.

11. KILLIAN, C. H. Etudes écologique sur la repartition du chlorure de sodium dans les psammophytes et halophytes algériens. Ann. Physiol. et Physico-Chim. Biol. **7**: 419–468. 1931.
12. MONTFORT, CAMILL. Physiologische und pflanzengeographische Seesalzwirkungen. I. Einfluss ausgeglichener Salzlösungen auf Mesophyll- und Schliesszellen: Kritik der Iljinschen Hypothese der Salzbeständigkeit. Jahrb. wiss. Bot. **65**: 502–550. 1926.
13. ————, and BRANDRUP, WOLFGANG. Physiologische und pflanzengeographische Seesalzwirkungen. II. Ökologische Studien über Keimung und erste Entwicklung bei Halophyten. Jahrb. wiss. Bot. **66**: 902–946. 1927.
14. ————, and ————. Physiologische und pflanzengeographische Seesalzwirkungen. III. Vergleichende Untersuchung der Salzwachstumreaktionen van Wurzeln. Jahrb. wiss. Bot. **67**: 105–173. 1927.
15. OSTERHOUT, W. J. V. On the importance of physiologically balanced solutions for plants. I. Marine plants. Bot. Gaz. **42**: 127–134. 1906.
16. ————. On the importance of physiologically balanced solutions for plants. II. Fresh water and terrestrial plants. Bot. Gaz. **44**: 259–272. 1907.
17. ————. The nature of balanced solutions. Bot. Gaz. **47**: 148–149. 1909.
18. ————. Quantitative criteria of antagonism. Bot. Gaz. **58**: 178–186. 1914.
19. ————. The measurement of antagonism. Bot. Gaz. **58**: 272–276. 1914.
20. ————. The forms of antagonism curves as affected by concentration. Bot. Gaz. **58**: 367–376. 1914.
21. ————. Normal and abnormal permeability. Amer. Jour. Bot. **2**: 93–94. 1915.
22. ————. Extreme alterations of permeability without injury. Bot. Gaz. **59**: 242–253. 1915.
23. ————. On the decrease of permeability due to certain bivalent kations. Bot. Gaz. **59**: 317–330. 1915.
24. ————. The effect of some trivalent and tetravalent kations on permeability. Bot. Gaz. **59**: 464–473. 1915.
25. ————. The determination of additive effects. Bot. Gaz. **60**: 228–234. 1915.
26. ————. Some aspects of selective absorption. Jour. Gen. Physiol. **5**: 225–231. 1922.

27. Raber, O. L. A quantitative study of the effect of anions on the permeability of plant cells. Jour. Gen. Physiol. **2**: 535–539; The antagonistic action of anions. Jour. Gen. Physiol. **2**: 541–544. 1920.

28. ————. A quantitative study of the effect of anions on the permeability of plant cells. II. Amer. Jour. Bot. **8**: 366–368. 1921.

29. ————. The effect of permeability on polyvalent cations in combination with polyvalent anions. Amer. Jour. Bot. **8**: 382–385. 1921.

30. ————. The effect upon the permeability of (I) the same substance as cation and anion and (II) changing the valency of the same ion. Amer. Jour. Bot. **8**: 464–470. 1921.

31. ————. Permeability of the cell to electrolytes. Bot. Gaz. **75**: 298–308. 1923.

32. Rikhter, A. A. Physiological basis for the tolerance of plants in southeast Russia. I. Halophytes. Jour. Exp. Landw. Südosten Russl. **3**: 3–16. 1927. (Biol. Abst. **2**: 339.)

33. Scarth, G. W. The penetration of cations into living protoplasm. Amer. Jour. Bot. **12**: 133–148. 1925.

34. Schimper, A. F. Pflanzengeographie auf physiologischer Grundlage. Jena. 1898.

35. St.John, H., and Courtney, D. The flora of Epsom Lake. Amer. Jour. Bot. **11**: 100–107. 1924.

36. Trelease, S. F., and Trelease, Helen M. Magnesium injury of wheat. Bull. Torrey Bot. Club **58**: 127–148. 1931.

37. True, R. H., and Bartlett, H. H. The exchange of ions between the roots of *Lupinus albus* and culture solutions containing one nutrient salt. Amer. Jour. Bot. **2**: 255–278. 1915.

38. ————, and ————. The exchange of ions between the roots of *Lupinus albus* and culture solutions containing two nutrient salts. Amer. Jour. Bot. **2**: 311–323. 1915.

39. Woronichin, N. N. Zur Biologie der bittersalzigen Seen in der Umgebung von Pjatigorsk (nördl. Kaukasus). Arch. Hydrobiol. **17**: 628–643. 1926.

40. Yapp, R. H. *Spiraea ulmaria* L. and its bearing on the problem of xeromorphy in marsh plants. Ann. Bot. **26**: 815–870. 1912.

41. Zellner, Julius. Zur Chemie der Halophyten. Sitzungsber. Akad. Wiss. Wien. Math. Nat.- Kl. Abt. I Ib. **135**: 585–592. 1926.

PHYSIOLOGICAL RÔLE OF ASPARAGINE AND RELATED SUBSTANCES IN NITROGEN METABOLISM OF PLANTS[1]

A. E. MURNEEK

Historical and theoretical retrospect

Asparagine, the amide of amino succinic acid, $COOH \cdot CHNH_2 \cdot CH_2 \cdot CONH_2$, seems to have been found in asparagus over one hundred years ago (**50**) and so named soon thereafter. MOTHES (**14**) credits HARTIG (1858) as having extracted this substance from seedlings and isolated it for the first time in crystalline form. He thought that asparagine was a translocation product of nitrogen in young plant tissues. BOUSSINGAULT (**2**), however, assigned to it a different function by expressing the belief that there is an analogy between asparagine and urea in nitrogen metabolism, both being amides and end products of protein degradation. But urea is excreted from the animal organism, while asparagine is not eliminated from plants. In presence of light, it is reutilized for the synthesis of proteins. These hypotheses of HARTIG and BOUSSINGAULT are still both supported and contended.

After extensive microchemical studies on the presence and absence of asparagine in all parts of a large number of plants, PFEFFER (1872) attempted to unite the two opposing views by concluding that: (1) Protein degradation in plants, because of formation of asparagine, differs from that in animals and from results obtained *in vitro*. (2) Asparagine is the primary, not the end product of protein breakdown and should be considered also a storage form of nitrogen. (3) Judging from its presence and solubility, it is the main translocation product of nitrogenous substances. It moves to growing regions, where it combines with carbohydrates (glucose) to form proteins. (Of interest in this respect is the fact that CHIBNALL (**4**) still seems to agree with PFEFFER that asparagine is the chief form in which nitrogen is translocated in plants).

SCHULZE (**36, 37, 38, 39, 42, 43, 44, 45**) for 30 years studied many phases of nitrogen metabolism in plants, with particular reference to asparagine, by physiological and chemical means. While he was unsuccessful in demonstrating an *in vivo* and *in vitro* parallelism of protein hydrolysis and asparagine accumulation, he did show definitely that under normal conditions asparagine is produced from proteins and not from inorganic nitrogen sources. But through further studies, largely with seedlings of legumes,

[1] Presented before the American Society of Plant Physiologists at the Symposium on Nitrogen Metabolism of Plants, Boston, December 30, 1933.

Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station, Journal Series no. 401.

SCHULZE and later his coworkers (primarily PRIANISCHNIKOV and associates) supplied evidence in support of the hypothesis that asparagine may be formed readily from ammonia and organic acids.

SCHULZE found that during early stages of germination and growth of seedlings, cotyledons contain only primary products of protein hydrolysis, the amino acids (leucine, tyrosine, etc.). During later development of the plant, amino acids disappear from the cotyledons and asparagine accumulates in large amounts in the shoots. Therefore asparagine does not seem to be produced directly from proteins,[2] but results from breaking down of the amino acids.

Through his own extensive investigations and those of many coworkers, PRIANISCHNIKOV (**20–31**) seems to have established the analogy between asparagine and urea in nitrogen metabolism. Moreover, he has demonstrated that asparagine may be synthesized directly in seedlings from substances within the plant and an external supply of ammonia or carbohydrates or both.

As a result of the preceding and many other studies, the following theoretical considerations seem to have become popular (**27, 6, 14,**):

> When, in comparison with carbohydrates, there is an excess of ammonia, or proteins are not required by the plant because of shortage of carbohydrates, then through hydrolysis of proteins by means of proteolytic enzymes amino acids are formed. Some of these (especially mono-amino acids) are oxidized and amino (NH_2) groups are released. Asparagine, which contains two NH_2 groups, is formed from two molecules of amino acids. One of these is oxidized to aspartic acid, the other much further with splitting off of ammonia. A union of aspartic acid with ammonia forms ammonium aspartate, from which, through dehydration, asparagine is produced in the same manner as in the animal organism urea is formed from carbamate of ammonia. When a plant is supplied with abundance of ammonia, asparagine may be formed from one molecule of an amino acid or even from an organic acid (malic, succinic, etc.). If there is need for nitrogen, asparagine will be broken down, NH_3 released, and the rest of the molecule may be oxidized to CO_2. Asparagine, therefore, has a dual function: removal of the injurious NH_3, and storage of N.
>
> In some plants, or under some conditions in most plants, not only asparagine but *glutamine*, the amide of amino-glutaric acid (**41**), *argi-*

[2] There is the possibility, of course, that asparagine may exist as such in the protein molecule, as is suggested by SCHULZE, OSBORN, and BUTKEWITSCH. In acid hydrolysis of proteins the amide group is saponified, giving aspartic or glutamic acids; but when proteins are acted upon by proteolytic enzymes, the amides appear unaltered. In many instances, however, the quantity obtained from seedlings is entirely too large to be accounted for on this basis.

nine, guanidine-amino-valerianic acid (**39, 40**), *allantoin*[3] or allantoic acid (**1**), *urea,* $CO(NH_2)_2$ (10), and possibly other substances may serve as receptors of NH_3. Organic acids (malic, succinic, oxalic) likewise may take care of ammonia by forming ammonium salts, which seem to be a characteristic feature of very acid plants.

When plants have an ample supply of carbohydrates (sugars) the process is reversed. Ammonia is released from any of the receptors and is used in the synthesis of amino acids, proteins, and other complex nitrogenous products.

Experimental evidence

No attempt, of course, is made here to present all the important evidence bearing on the subject under discussion. Only some of the typical results of investigations on certain parts of the major phases of the problem will be recounted. Those desiring to secure more complete information will naturally turn to the extensive literature in this field.

1. Nitrogen metabolism of seedlings

Changes in nitrogenous substances in the germinating seeds and seedlings have been thoroughly investigated by Schulze, Prianischnikov, and others. This has involved a study of the breakdown of proteins in the cotyledons and regeneration of nitrogen compounds in the developing stem and leaves. A conspicuous feature, often emphasized in these studies, is the abundance of asparagine in the seedlings of legumes, especially when grown in darkness. It is evident from Schulze's and other work (**4**) that with increased development of the plant there is a decrease in protein concentration and a concomitant increase in asparagine and mono-amino acids (table I). But since the amino acid fraction decreases when the seedlings

TABLE I

Nitrogen metabolism in legume seedlings (Schulze)

Age of seedlings in days	Protein	Asparagine	Other N (mostly mono-amino acids)
	%	%	%
6	5.49	1.16	1.72
12	1.71	4.02	2.39
24	1.78	5.09	1.40

[3] Allantoin, $CO\begin{<}\begin{matrix}NH\text{–}CHNH.CONH_2\\ |\\ NH\text{–}CO\end{matrix}$, a diuride of glyoxalic acid.

are of considerable age, while at the same time there is a proportional increase in asparagine and no further decrease in proteins, it appears that asparagine may have come from the amino acid fraction. These plants, of course, had no external supply of nitrogen.

Similar results have been obtained not only with several legumes but also with other seedlings by many workers. Asparagine accumulates likewise in onion bulbs, asparagus roots, and similar plant structures when they are sprouted in darkness (**54, 18, 32**).

The converse of this process takes place when, for instance, seedlings are grown in light and proteins are synthesized in presence of sufficient amount of carbohydrates, or in case of development of bulbs, whose sugar content is usually high (**54, 56**). In general, the higher the amount of soluble carbohydrates in the seeds, bulbs, or roots, the less protein will be broken down owing to respiration, when growth is resumed, and there will be relatively little fluctuation in asparagine concentration.

Most oil-containing seeds produce upon germination glutamine (**41**) instead of asparagine, but the general nitrogen transformations seem to be the same. In some plant seedlings other substances than acid amides appear to play a similar rôle, as will be noted further on.

2. Nitrogen metabolism in leaves

Several investigators have demonstrated that asparagine and its homologue, glutamine, perform the same function in nitrogen metabolism of leaves as in developing seedlings. In young rapidly growing leaves, both amides may be used in synthesis of proteins (**44**). In fully developed leaves proteins are formed during the day and may be broken down and removed in soluble forms of N to other parts of the plant. Chibnall (**4, 5**), for example, has demonstrated that asparagine disappears from the leaves of the bean plant at night and that it comes largely from hydrolysis of proteins. In fruit-bearing plants nitrogen may be translocated in this form to the fruit or seed pods whence eventually it will go into the seeds (**44, 45, 53**).

Alterations in nitrogen content of starved or senescent leaves supply additional evidence of the rôle of acid amides in plant metabolism. The results secured by Miyachi are presented here (table II). They show that when leaves are starved there is a rapid breakdown of proteins and a striking increase in asparagine and other amides. Similarly, Vickery (**52**) has found that during the curing of tobacco leaves, with increasing loss of amino-N there is a more or less corresponding gain in amide-N (table III). He thinks that such change may be due to the oxidation of amino acids, the formation of ammonia, and subsequent synthesis of amides. As a result of these changes, ammonia was maintained at a relatively low level.

TABLE II

AMIDE PRODUCTION IN STARVED LEAVES OF PEONY (MIYACHI)

NITROGEN	FRESH LEAVES	LEAVES STARVED 15 DAYS
	%	%
Total	1.364	1.462
Protein	1.312	0.801
Asparagine	0.037	0.206
Other amides	0.015	0.455
	PERCENTAGE OF TOTAL N	
Protein	96.19	54.79
Asparagine	2.71	14.09
Other amides	1.10	31.12

The formation of amides, therefore, should be considered as a "defensive mechanism" for the detoxication of NH_3, which comes into operation when there is a rapid destruction of proteins and accumulation of NH_3, or when there is an excessive reduction of NO_3 to NH_3.

Probably the most extensive and complete studies of nitrogen changes in leaves have been conducted by MOTHES (**14, 15**). After investigating a series of plants that had been subjected to various environments and experimental treatments, he draws the following conclusion: When leaves are exposed to light or are fed with glucose in darkness, no amides are formed and even the original amount disappears by being used in synthesis of proteins. But when the carbohydrate content of the leaves is reduced to a low point, as a result of long exposure to darkness, then amides are produced promptly and eventually ammonia appears. Oxygen seems to be a

TABLE III

CHANGES IN AMINO ACIDS AND AMIDES IN CURING TOBACCO LEAVES (VICKERY)

TIME	AMINO–N LOSS	AMIDE–N GAIN
hr.	*gm.*	*gm.*
41	0.020	−0.005
64	0.015	−0.017
87	0.370	0.019
111	0.007	0.018
159	0.110	0.278
183	0.395	0.318
207	0.290	0.449
231	0.515	0.546
279	0.820	0.835
303	0.290	0.446

limiting factor in amide formation. Without its presence, neither amides nor ammonia will be produced even in leaves of a low carbohydrate content, but there will be an increase of amino, basic, and other ("rest-N") nitrogen fractions.

Oxidation of the products of hydrolysis of proteins takes place in leaves when there is a shortage of carbohydrates, the final end product being ammonia. In presence of some carbohydrates, ammonia is "neutralized" by being used for the formation of asparagine, in which form NH_3 is "stored." Whether produced within the plant or supplied from an external source, asparagine will remain as such unless carbohydrates become available, when it enters promptly into synthesis of proteins. So, too, when ammonium salts are fed to leaves high in carbohydrates, proteins will be formed quickly; when carbohydrates are short, asparagine will be produced; and when carbohydrates are absent, ammonia will accumulate in the cells until poisoning of the leaves occurs. In this respect, ammonia supplied from without behaves physiologically the same as NH_3 released within the plant. When leaves with ample carbohydrate supply are exposed to narcotics, no amides and often no ammonia will be produced.

These conclusions are in striking agreement with the results of other investigators, as the succeeding discussion will show. MOTHES seems to have verified with mature plants most of the evidence obtained with seedlings.

3. SOME FACTORS AFFECTING ASPARAGINE FORMATION

While the procedure and mechanism of accumulation and disappearance of asparagine and related substances in plant metabolism are not yet understood in all their details, we know that several factors seem to affect them. Some of these will be discussed briefly.

a. IMPORTANCE OF OXIDATION.—Proteins may be decomposed in plants with or without the presence of oxygen, the usual hydrolysis products being the amino acids (**19**). In the presence of oxygen, however, asparagine (or glutamine) often appears as the main soluble N substance. With further oxidation it is broken down to NH_3. That oxidation is really essential for the production of amides and ammonia has been demonstrated, among others, by SUSUKI (**49**) with barley (table IV) and soy beans, and by BUTKEWITSCH (**3**) with lupine (table V). SUSUKI found not only an increase in asparagine when seedlings were developed in darkness in presence of oxygen, but also a marked decrease of amino acids. This has been corroborated by WASSILIEFF (**53**). Without a supply of oxygen, amides are not formed and amino acids accumulate. With further oxidation and absence of carbohydrates, ammonia is produced in large quantities owing to the breakdown (oxidation) of asparagine (table V). SURE and TOTTING-

TABLE IV

EFFECT OF O_2 ON ASPARAGINE FORMATION IN BARLEY SEEDLINGS (SUSUKI)

	DRY MATTER	PER 100 SEEDLINGS
	%	*gm.*
At beginning of experiment	6.59	0.1427
After 45 hours in darkness with O_2	8.85	0.1818
After 45 hours in darkness without O_2	7.16	0.1336

TABLE V

EFFECT OF O_2 ON FORMATION OF AMMONIA AND AMIDE-N IN GERMINATING SEEDS OF LUPINE (BUTKEWITSCH)

NITROGEN	PER 100 SEEDLINGS	
	WITHOUT O_2	WITH O_2
	mg.	*mg.*
Ammonia	19.38	170.60
Amide	171.36	139.48
Ammonia and amide	190.74	310.08

HAM (**47**) have shown also that in the shoots of pea seedlings there is a simultaneous decrease of α-amino acids and ammonia, which indicates that "α-amino acids serve for amide production in the nitrogen metabolism of the etiolated pea plant."

b. EFFECTS OF ANAESTHETICS.—Since the time when CLAUDE BERNARD (1878) demonstrated that anaesthetics inhibit anabolism but permit catabolism, various experiments have been performed to show their effects on N metabolism in plants. BUTKEWITSCH (**3**), for example, exposed seedlings to fumes of toluol with the result that no asparagine was formed but instead large amounts of NH_3 accumulated, up to 14 per cent. of total N. This suggests that asparagine arises not as a result of tearing down but through a building up or synthetic process.

c. EFFECTS OF PRESENCE OF CARBOHYDRATES.—It has already been noted that with extreme carbohydrate deficiency the carbon structure of the asparagine molecule is broken down (oxidized) and NH_3 accumulates. Synthesis of asparagine may be effected by means of an external supply of glucose, as table VI shows. An artificial supply of sugar will not only diminish the accumulation of NH_3 but will result in a marked increase of amides (asparagine) (**3, 46**), which eventually will lead to the formation of proteins (**49, 46**). Several investigators have found that asparagine disappears when seedlings, grown in darkness, are supplied with sugar

TABLE VI

EFFECT OF GLUCOSE ON AMMONIA AND AMIDE-N CONCENTRATION IN SEEDLINGS OF LUPINE (BUTKEWITSCH)

NITROGEN	WITHOUT GLUCOSE	WITH GLUCOSE
	%	%
Ammonia	18.57	9.37
Amide	15.17	23.10

(**48, 46**). Contrariwise, an abundance of sugar prevents asparagine formation from proteins, but under the right conditions it does not interfere with and may even stimulate the synthesis of this amide.

One may conclude with PRIANISCHNIKOV (**26**), therefore, that with carbohydrate shortage, proteins will be hydrolyzed and there will be formed amino acids of the general formula $R(NH_2)COOH$, which, through oxidation and secondary synthesis, produce acid amides of the general formula $R(NH_2)CONH_2$ (asparagine and glutamine), and, in case of carbohydrate starvation, give, through further oxidation, NH_3. But when the carbohydrate supply is abundant, whatever its source, then the reverse process takes place. From NH_3 to acid amides ($R(NH_2)CONH_2$), which, with additional supply of carbohydrates, give rise to amino acids ($R(NH_2)$-$COOH$), and these in turn synthesize proteins.

The frequently observed results of formation of asparagine in light and not in darkness are due not to the direct effect of light but to the presence of carbohydrates in light, as was demonstrated by PRIANISCHNIKOV (**28**), who gives the schematic summary shown in table VII.

d. SYNTHESIS OF ASPARAGINE FROM AMMONIUM SALTS.—One of the crucial experiments in the determination whether asparagine originates directly from breakdown of proteins or comes from the synthesis of NH_3 with other oxidation products of proteins, is through feeding plants in various states of carbohydrate deficiency with ammonium salts.

TABLE VII

CARBOHYDRATE AND LIGHT RELATIONSHIPS IN THE FORMATION OF ASPARAGINE AND AMMONIA (PRIANISCHNIKOV)

EXPERIMENTAL CONDITIONS		RESULTS	
CARBOHYDRATES	LIGHT	ASPARAGINE SYNTHESIS	AMMONIA INJURY
+	−	+	−
−	−	−	+
+	+	+	−
−	+	−	+

Ever since SUSUKI (**48**), HANSTEEN (**7**), and ZALESKI (**55**) demonstrated that proteins may be formed from various nitrogenous substances supplied to the plants, much work has been done with the object of determining to what extent and under what circumstances asparagine is synthesized from N constituents of nutrient solutions. Thus PRIANISCHNIKOV (**24, 25, 26, 29**) and others (**48, 46**) have shown that by supplying salts of nitrogen to certain seedlings their asparagine content is markedly increased. For this purpose ammonia is a better source of N than the nitrates, when the physiological acidity of ammonium salts is neutralized by a base ($CaCO_3$, etc.) (**48, 25**). The legumes in particular seem to require Ca to neutralize the acid reaction of NH_4Cl and $(NH_4)_2SO_4$, which appear to inhibit the formation of asparagine (table VIII). Barley and corn seedlings, high

TABLE VIII

RESULTS OF FEEDING VETCH SEEDLINGS WITH AMMONIUM SALTS WITH AND WITHOUT CALCIUM (PRIANISCHNIKOV)

AMOUNT N IN 100 SEEDLINGS	DISTILLED H_2O	NH_4Cl	$NH_4Cl + CaCO_3$
	mg.	*mg.*	*mg.*
Protein N	85	109	90
Asparagine N	75.9	73.9	118.2
Ammonia N	0.9	0.9	1.0

in carbohydrates, will absorb NH_3 from these salts equally well either with or without the presence of calcium. But when carbohydrates are exhausted (darkness) in corn or barley seedlings, they will behave like legumes. And legumes can be put, by various methods, into a physiological state similar to that of cereal seedlings (table IX.)

TABLE IX

RELATION OF NITROGEN TO CARBOHYDRATES IN VARIOUS TYPES OF SEEDLINGS (PRIANISCHNIKOV)

GRAMINEAE	STARCH CONTAINING LEGUMES	LUPINE
1: 6	1: 2.0 – 2.5	1: 0.6

The importance of Ca ions in this respect is not clearly understood, for this ion increases even the utilization of N from nitrates (**25**). Very likely calcium has something to do with the carbohydrate metabolism. It of course neutralizes to some extent the acidity of the nutrient medium, since cations (NH_4) are absorbed faster than anions. PRIANISCHNIKOV thinks that Ca ions increase respiration and hydrolysis of proteins.

Lupine seedlings with very small carbohydrate reserves are unable to assimilate N from either NH_4Cl or $(NH_4)_2SO_4$, even in presence of Ca salts. But when exposed to light or fed glucose, they will utilize N under the above conditions. Similarly barley seedlings, after prolonged growth in darkness and loss of all starch reserves, are incapable of assimilating nitrogen. When in this state they behave like lupine seedlings. Carbohydrates and the essential internal environmental factors, therefore, are necessary for the synthesis of asparagine from inorganic nitrogen.

e. Synthesis of asparagine from NH_3 and organic acids.—Because of the similarity in chemical structure between some of the organic acids and acid amides, it has long been suspected that they may be interrelated. Moreover, evidence has accumulated in support of the view that organic acids are produced when proteins are broken down (**12, 34, 35**), although very likely they may be formed also from sugars (**14**). Malic and succinic acids seem to be the two that, in presence of ammonia, participate readily in the synthesis of asparagine. By feeding corn seedlings ammonium sulphate and ammonium salts of organic acids, Smirnov (**46**) was able to demonstrate that both malic and succinic acids enter into the synthesis of asparagine (table X), and that, in presence of glucose, eventually proteins accumulate (table XI).

TABLE X

Results of feeding corn seedlings ammonia with and without organic acids (Smirnov)

Form of ammonia	Percentage of total N			
	Protein N	Ammonia N	Asparagine N	Amino acid N
	%	%	%	%
$(NH_4)_2SO_4$	66.62	4.19	13.34	15.85
Ammonium malate	58.42	3.87	19.30	18.41
Ammonium succinate	63.57	3.33	16.16	16.94

TABLE XI

Protein synthesis in corn from asparagine in presence of glucose (Smirnov)

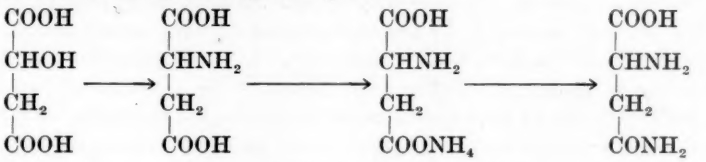
Source of ammonia	Percentage of total N		
	Protein N	Ammonia N	Asparagine N
	%	%	%
$(NH_4)_2SO_4$	66.79	4.4	15.10
Ammonium succinate	67.75	4.2	19.52
Ammonium aspartate	70.25	4.5	17.6

Many investigators (PRIANISCHNIKOV etc.) conceive the path of formation of asparagine from malic acid through aspartic acid and ammonium aspartate (table XII).

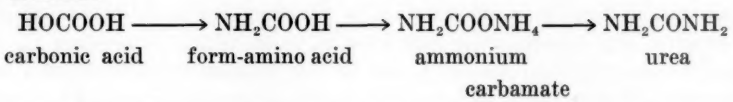
TABLE XII

ANALOGY BETWEEN ASPARAGINE AND UREA

Probable formation of asparagine in plants:



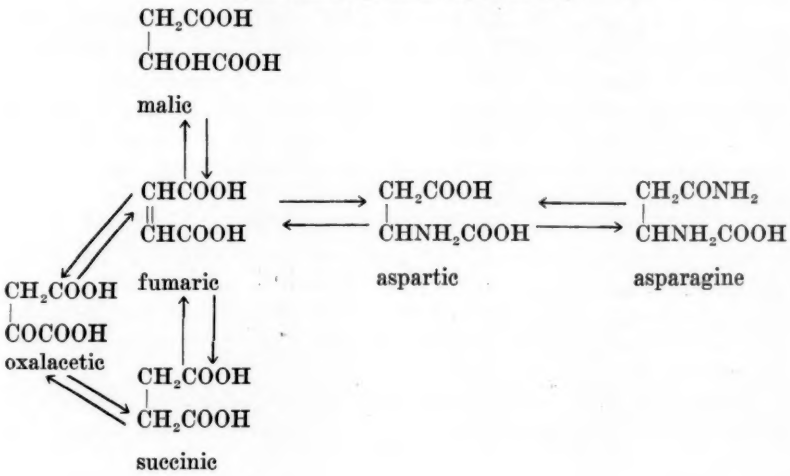
In animals:



Based on ROBINSON'S (**33**) ideas, VICKERY and PUCHER (**52**) present an additional plan, which shows the connection between asparagine and α-keto and hydroxy acids (table XIII). They state that "this purely hypothetical scheme involves, however, only reactions of known biological significance."

TABLE XIII

ROBINSON'S SCHEME SHOWING CONNECTION BETWEEN ASPARAGINE AND α-KETO AND HYDROXY ACIDS (VICKERY AND PUCHER)



Another more direct proof that ammonium salts of organic acids, although participating in the synthesis of proteins, are not so easily assimilated as asparagine, comes from experiments of feeding plants with these substances. NAKAMURA (**16, 17**), for instance, found that both phanerogams and fungi utilize more readily asparagine than ammonium succinate from a weak nutrient solution. By feeding asparagine to leaves of high carbohydrate content, PRIANISCHNIKOV and SMIRNOV were able to demonstrate protein synthesis. When carbohydrates were absent, asparagine remained intact in the leaves. This has been corroborated by NAKAMURA with barley seedlings. Hence there seems to be considerable proof in support of the above path of utilization of asparagine in protein formation.

f. ORGANIC ACIDS AS DIRECT RECEPTORS OF AMMONIA.—Recently RUHLAND and WETZEL (**34, 35**) have shown that in some highly acid plants (*Oxalis, Begonia, Rheum*) oxalic acid takes care of ammonia by forming ammonium oxalate and possibly ammonium salts of other acids. Amides are not present in any appreciable quantities in these plants, excepting in the older leaves, which are less acid. In *Rheum* both amides and organic ammonium salts may serve as receptors of NH_3 in various organs of the same plant, depending on their relative acidity. They conclude, therefore, that amides are formed in less acid plants (or tissues) and organic salts of ammonia in more acid ones.

There is much information on record showing a connection between organic acids and protein transformation. Malic, tartaric, citric, and other acids may come either from amino acids or from intermediary products of protein disintegration. But a strongly oxidized acid, like oxalic, can be derived from sugars as well as proteins.

Continuing RUHLAND and WETZEL's investigations, KULTZSCHER (**13**) found that very acid plants ($pH<5.00$) are able to store large quantities of excess nitrogen in the form of ammonium salts of organic acids. Their function is not merely a case of neutralization of NH_3 but also of storage of N. In deamination of amino acids not only NH_3 is released but also acids are formed, which then more or less automatically take care of each other by forming ammonium salts.

According to KULTZSCHER, it would seem that an equilibrium exists between amides and ammonia. In plants relatively high in actual (pH) and potential acidity, the equilibrium is shifted to the NH_3 side and ammonium salts are formed through the union of NH_3 with organic acids. The fact is emphasized by KULTZSCHER that highly acid plants are characterized by an active deamination process.

Ammonium salts of oxalic and other organic acids will take care of proportionally larger quantities of NH_3 than asparagine does, but urea of

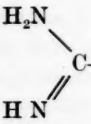
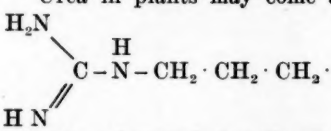
still more, and guanidine,  , most of all the compounds so far found in plants. The ratios of carbon to nitrogen in various substances that may act as receptors of nitrogen are presented in table XIV.

TABLE XIV

RATIO OF CARBON TO NITROGEN IN VARIOUS ORGANIC SUBSTANCES WHICH MAY ACT AS NITROGEN RECEPTORS IN PLANTS

	C : N
Glutamine	2.5 : 1
Asparagine	2 : 1
Ammonium malate or tartrate or succinate or citrate	2 : 1
Arginine	1.5 : 1
Ammonium oxalate	1 : 1
Allantoin	1 : 1
Urea	1 : 2
Guanidine	1 : 3

g. FORMATION OF UREA AND OTHER MEANS OF REMOVING NH_3.—Many fungi are capable of forming and even absorbing from without large quantities of urea, $CO(NH_2)_2$, but as a rule do not excrete it. According to IWANOV (**8**) some fungi (*Lycoperdon, Bovista*) may accumulate half of the total N as urea (up to 11 per cent. of dry weight). Fungi will absorb urea and thiourea from weak solutions of these substances and store it up to 15 per cent. of their dry weight (**9**).

Urea in plants may come also from the breaking down of arginine,  which itself may act as an NH_3 receptor. Ammonia is split off easily from arginine by the enzyme arginase (**11**). Arginine is supposed to have the same function in conifers as asparagine in other plants.

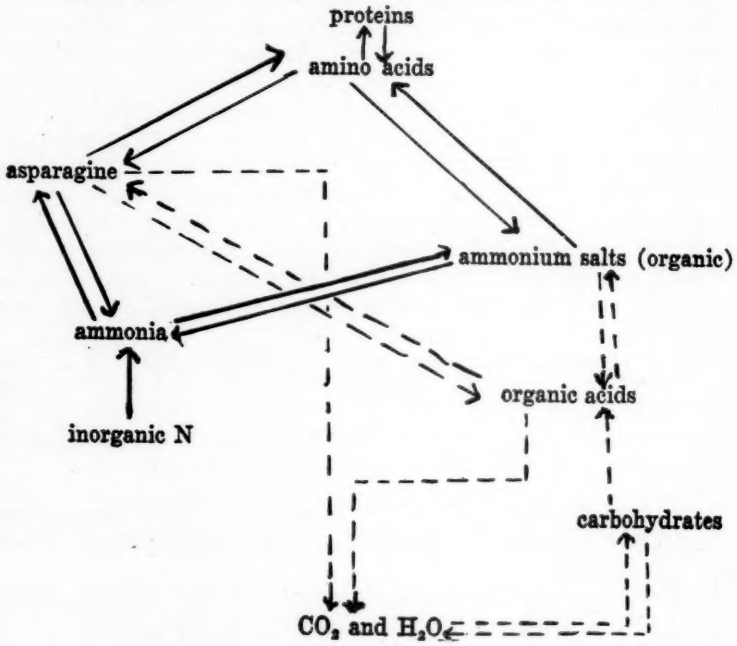
Whatever its source, urea seems to accumulate in some plants in absence of carbohydrates (**10**), and since it is used up in certain stages of the development of the organism, it should be considered as a storage form of N, and therefore analogous to asparagine and glutamine in its physiological function. Moreover, urea may be changed to asparagine. According to SUSUKI (**48**) and PRIANISCHNIKOV (**25**), it is frequently a better nutrient form of N than ammonium salts for the formation of asparagine.

From the preceding discussion it is apparent that there are at the disposal of plants various means of removing and neutralizing NH_3. The mechanism set into operation in each instance may possibly depend upon several factors, of which the most important seems to be the carbohydrate content of the plant as a whole or of specific organs, the hydrogen ion concentration and the source (meaning the rest of the molecule) from which NH_3 comes. Doubtless many environmental factors also play a rôle in this respect. Still one must be mindful of the fact that under extreme conditions of carbohydrate depletion, nitrogen may be excreted from the plant through the roots in the form of ammonia (**30, 31**).

A generalized scheme of nitrogen metabolism in plants, modified after ENGEL (**6**), is presented in table XV.

TABLE XV

SCHEME OF N METABOLISM IN HIGHER PLANTS (MODIFIED AFTER ENGEL)



4. ANALOGY BETWEEN ASPARAGINE IN PLANTS AND UREA IN PLANTS AND ANIMALS

Both amides, asparagine and urea, do not seem to be the direct products of protein hydrolysis but arise from secondary synthesis with NH_3 as the

key ion, which most often comes from oxidation of amino acids. Both amides may be synthesized in plants when NH_3 is introduced into the organism, with this difference between the two, that for synthesis of asparagine a part of the unoxidized carbohydrate molecule is necessary while for formation of urea NH_3 and CO_2 are sufficient (table XII). The physiological function of both processes appears to be the neutralization and storage of NH_3, which seems to be toxic to living organisms.

The analogy between plants and animals in this respect is that neither of them can synthesize the respective amides from ammonium salts of strong acids, but more neutral salts of ammonia will lead to such synthesis.

There is a great difference, however, in respect to the further metabolic rôle of the two amides. Urea is excreted from animals. They do not need to be so economical with N, for its intake through feeds is more or less assured and there is no caloric value in CO_2, the carbon part of the molecule. Asparagine, on the other hand, remains in the cells of plants as a reserve substance of N, which, with renewed supply of carbohydrates, can be used again for the production of amino acids and other components of proteins.

COLLEGE OF AGRICULTURE
UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI

LITERATURE CITED

1. BONNET, R. L'évolution de l'azote au cours de la germination. Bull. Soc. Chem. Biol. **11**: 1025–1061. 1929.
2. BOUSSINGAULT, M. De la végétation dans l'obscurité. Compt. Rend. Acad. Sci. Paris **58**: 917–922. 1864.
3. BUTKEWITSCH, W. Das Ammoniak als Umwandlungsprodukt stickstoffhaltiger Stoffe in höheren Pflanzen. Biochem. Zeitschr. **16**: 411–452. 1909.
4. CHIBNALL, A. C. Investigations in the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 344–362. 1922.
5. ————. VI. The rôle of asparagine in the metabolism of the mature plant. Biochem. Jour. **18**: 395–404. 1924.
6. ENGEL, H. Beiträge zur Kenntnis des Sticksoffumsatzes grüner Pflanzen. Planta **7**: 133–164. 1929.
7. HANSTEEN, B. Ueber Eiweisssynthese in grünen Phanerogamen. Jahrb. wiss. Bot. **33**: 417–486. 1899.
8. IWANOV, N. N. Über den Harnstoffgehalt der Pilze. Biochem. Zeitschr. **136**: 1–19. 1923.
9. ————. Absorption des Harnstoffs durch Pilze. Biochem. Zeitschr. **150**: 115–122. 1924.

10. ————————. Der Harnstoff der Pilze und dessen Bedeutung. Zeitschr. physiol. Chem. **170**: 274–288. 1927.
11. KLEIN, G., and TAUBÖCK, K. Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. Biochem. Zeitschr. **255**: 278–286. 1932.
12. KOSTYTCHEV, S., and FREY, L. Über Alcoholgärung. XI. Über die bei der Hefegärung in Gegenwart von Calciumcarbonat entstehenden Säuren. Zeitschr. physiol. Chem. **146**: 276–285. 1925.
13. KULTZSCHER, M. Die biologische NH_3-Entgiftung in höheren Pflanzen in ihrer Abhängigkeit von der Wasserstoffionen-Konzentration des Zellsaftes. Planta **17**: 699–757. 1932.
14. MOTHES, K. Ein Beitrag zur Kenntnis des N-Stoffwechsels höherer Pflanzen. Planta **1**: 472–552. 1926.
15. ————————. Zur Kenntnis des N-Stoffwechsels höherer Pflanzen. 3. Beitrag. Planta **12**: 686–731. 1931.
16. NAKAMURA, T. On the relative value of asparagine as a nutrient of phanerogams. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 465–467. 1897.
17. ————————. On the relative value of asparagine as a nutrient for fungi. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 468–470. 1897.
18. NIGHTINGALE, G. T., and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
19. PALLADIN, W. Ueber Zersetzungsproducte der Eiweisstoffe in den Pflanzen bei Abwesenheit von freiem Sauerstoff. Ber. deutsch. bot. Ges. **6**: 296–304. 1888.
20. PRIANISCHNIKOV, D. Zur Kenntnis der Keimungsvorgänge bei *Vicia sativa*. Landw. Vers.-Sta. **45**: 247–288. 1895.
21. ————————. Eiweisszerfall und Eiweissrückbildung in den Pflanzen. Ber. deutsch. bot. Ges. **17**: 151–155. 1899.
22. ————————. Die Rückbildung der Eiweisstoffe aus deren Zerfallsprodukten. Landw. Vers.-Sta. **52**: 347–381. 1899.
23. ————————. Zur Frage der Asparaginbildung. Ber. deutsch. bot. Ges. **22**: 35–43. 1904.
24. ————————. La synthèse des corps amidés aux dépens de l'ammoniaque absorbée par les racines. Rev. gén. Bot. **25**: 5–13. 1913.
25. ————————. Das Ammoniak als Anfangs- und Endprodukt des Stickstoffumsatzes in den Pflanzen. Landw. Vers.-Sta. **99**: 267–286. 1922.
26. ————————. Über den Aufbau and Abbau des Asparagins in den Pflanzen. Ber. deutsch. bot. Ges. **40**: 242–248. 1922.

27. ————. Asparagin and Harnstoff. Biochem. Zeitschr. **150**: 407–423. 1924.
28. ————. Sur le rôle de l'asparagine dans les transformations des matières azotées chez les plantes. Rev. gén. Bot. **36**: 108–122; 159–181. 1924.
29. ————, and SCHULOV, J. Über die synthetische Asparaginbildung in den Pflanzen. Ber. deutsch. bot. Ges. **28**: 253–264. 1910.
30. ————, and IWANOV, V. Absorption and excretion of ammonia by roots. Compt. Rend. Acad. Sci. U.S.S.R. **1929**: 327–331. (In Russian).
31. ————, and ————. Formation of ammonia during the reduction of nitrates in higher plants. Compt. Rend. Acad. Sci. U.S.S.R. **1931**: 205–209. (In Russian).
32. RAHN, H. Untersuchungen über den N-Stoffwechsel pflanzlicher vegetativer Speicherorgane. Planta **18**: 1–51. 1932.
33. ROBINSON, MURIEL E. The protein metabolism of the green plant. New Phytol. **28**: 117–149. 1929.
34. RUHLAND, W., and WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. I. *Begonia semperflorens*. Planta **1**: 558–564. 1926.
35. ————, and ————. Zur Physiologie der organischen Säuren in grünen Pflanzen. III. *Rheum hybridum* Hort. Planta **3**: 765–769. 1927.
36. SCHULZE, E. Ueber Zersetzung und Neubildung von Eiweissstoffen in Lupinenkeimlingen. Landw. Jahrb. **7**: 411–444. 1878.
37. ————. Ueber den Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. **9**: 689–748. 1880.
38. ————. Ueber die Bildungsweise des Asparagins und über die Beziehungen der stickstoff freien Stoffe zum Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. **17**: 683–711. 1888.
39. ————. Über das Vorkommen von Arginin in den Wurzeln und Knollen einiger Pflanzen. Landw. Vers.-Sta. **46**: 451–458. 1896.
40. ————. Ueber die beim Umsatz der Proteïnstoffe in den Keimpflanzen einiger Coniferen-Arten enstehenden Stickstoffverbindungen. Zeitschr. physiol. Chem. **22**: 435–448. 1896.
41. ————. Uber die Verbreitung des Glutamins in den Pflanzen. Landw. Vers.-Sta. **48**: 33–55. 1897.
42. ————. Ueber den Umsatz der Eiweissstoffe in der lebenden Pflanze. Zeitschr. physiol. Chem. **24**: 18–114. 1898.

43. ————————. Über den Abbau und den Aufbau organischer Stickstoffverbindungen in den Pflanzen. Landw. Jahrb. **35**: 621–666. 1906.
44. ————————. Studien über die Proteinbildung in reifenden Pflanzen. Zeitschr. physiol. Chem. **71**: 31–48. 1911.
45. ————————, and WINTERSTEIN, E. Studien über die Proteinbildung in reifenden Pflanzensamen. Zeitschr. physiol. Chem. **65**: 431–476. 1910.
46. SMIRNOV, A. I. Über die Synthese der Säureamide in den Pflanzen bei Ernährung mit Ammoniaksalzen. Biochem. Zeitschr. **137**: 1–34. 1923.
47. SURE, B., and TOTTINGHAM, W. E. The relation of amide nitrogen to the nitrogen metabolism of the pea plant. Jour. Biol. Chem. **26**: 535–548. 1916.
48. SUSUKI, U. On the formation of asparagine in plants under different conditions. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 409–457. 1897.
49. ————————. On the formation of asparagine in the metabolism of shoots. Japanese Imp. Univ. Coll. Agr. Bull. **4**: 351–356. 1902.
50. VICKERY, H. B. Some nitrogenous constituents of the juice of the alfalfa plant. VI. Asparagine and amino acids in alfalfa. Jour. Biol. Chem. **65**: 657–664. 1925.
51. ————————, *et al.* Chemical investigations of the tobacco plant. Carnegie Inst. Publ. no. 445. 1933.
52. ————————, and PUCHER, G. W. The chemical changes that occur during the curing of Connecticut shade-grown tobacco. Connecticut Agr. Exp. Sta. Bull. 324. 1931.
53. WASSILIEFF, N. Eiweissbildung in reifenden Samen. Ber. deutsch. bot. Ges. **26a**: 454–467. 1908.
54. ZALESKI, W. Zur Keimung der Zwiebel von *Allium cepa* und Eiweissbildung. Ber. deutsch. bot. Ges. **16**: 146–151. 1898.
55. ————————. Zur Aetherwirkung auf dien Stoffumwandlung in den Pflanzen. Ber. deutsch. bot. Ges. **18**: 292–296. 1900.
56. ————————, and SHATKIN, W. Untersuchungen über den Eiweissaufbau in den Pflanzen. I. Über den Eiweissaufbau in den Zwiebeln von *Allium cepa*. Biochem. Zeitschr. **55**: 72–78. 1913.

FACTORS INFLUENCING THE GROWTH AND YIELD OF POTATOES IN FLORIDA

M. R. ENSIGN

(WITH NINE FIGURES)

Introduction

It is common knowledge that the early Irish potato crop in Florida shows wide fluctuations in yields from year to year; plantings made at various times during any one year also show as erratic variations. It is the consensus of opinion among Hastings and LaCrosse growers that the plantings made after the middle of January have usually produced larger average yields than those planted prior to that time. At present, the time of planting in the Hastings area[1] begins about the first part of December and extends to the first week in February. Plantings at LaCrosse usually do not begin prior to January 10.

A series of time-of-planting tests was begun in 1927 and continued until the harvesting of the 1931 crop. The object of these experiments was to determine, if possible, some of the factors influencing the growth and yield of potatoes.

Review of literature

The literature dealing with potato experiments shows that rainfall (soil moisture) and temperature exert considerable influence upon growth and yield. The irrigation experiments of WIDTSOE (**8, 9, 10**) and HARRIS (**4, 5**) of Utah were especially significant since similar results were obtained from repetitions extending over two 5-year intervals. Their results showed that the middle period of growth, about the time of blossoming and tuber setting, was the most sensitive to moisture variations as reflected by the yield; if but one irrigation was given during the growing season, the best results were obtained when this was applied during the middle period of growth. SMITH (**7**) obtained similar results when he compared the rainfall with the potato yields in Ohio over a 50-year period. He too found a 30–40-day period about the time of blossoming to be the most sensitive to water requirements.

The work of BUSHNELL (**1**) showed that as temperatures rose above 68° F. (20° C.) the yield of potatoes decreased, and when grown at a temperature of 86° F. practically no tubers were formed, since respiration was so rapid as to consume the food as soon as it was manufactured. HARDENBURG (**3**)

[1] Federal Point is included in the Hastings area, and the earliest plantings begin there, since it enjoys some frost protection on account of its proximity to the St. Johns River.

grew potatoes in two greenhouses, in one of which he maintained an approximate temperature of 60° F. while the other was operated at a temperature 8° higher. The larger yield was produced at the lower temperature.

Numerous other investigations corroborate these essential facts, namely: (1) soil moisture, measured in inches of rainfall or acre inches of irrigation, plays an important part in the growth and yield of potatoes, and yields are most noticeably influenced by variations in available water during the middle period of growth; (2) temperatures above 68° F. during the growing period restrict the yield of potatoes.

Methods of experimentation

The time-of-planting tests were begun on January 10, 1927, and continued for five consecutive years. Plantings were made only at LaCrosse the first year; at Hastings, as well as LaCrosse, the second year; and at Hastings only the last three years. The tests at LaCrosse were discontinued after 1928 because of wide soil variations and because of the presence of a soil-borne disease, *Rhizoctonia,* that interfered with the interpretation of results.

Plantings were made at weekly intervals the first two years, beginning January 10 and ending February 20. It then became evident that the planting range should be extended to include two other periods, mid-December and January 1. These dates were therefore included in the planting schedule of the last three years of the experiment.

The prevailing soil type on which the tests were conducted both at LaCrosse and Hastings is commonly called flatwoods, and is classified as Bladen, consisting of a sandy loam with a clay subsoil 12–18 inches below the surface which tends to insure a fairly satisfactory and constant soil moisture content. The water saturation point of these soils was slightly above 22 per cent.; the wilting point about 1 per cent., based upon oven dryness of samples from the first foot of soil; the average pH was 4.78. The fields used for the tests had been almost continuously cropped to potatoes for ten years, with corn and cowpeas or peanuts as summer crops.

A ready-mixed fertilizer made up of 5 per cent. available nitrogen, 7 per cent. phosphoric acid, and 5 per cent. potash was distributed at the rate of 2000 pounds per acre in the drill two weeks prior to planting each lot of seed.

Only Maine certified seed of the Spaulding Rose variety was used, the same source being adhered to in all the plots each year. The seed was cut by hand the day prior to planting and the pieces were made as uniform in size as practicable, averaging about 1.5 oz. each. Copper lime dust was applied at 7–10-day intervals after the plants were 4–6 inches high, to combat disease. Late Blight was the most serious disease in the Hastings area,

although Early Blight was not altogether absent. *Rhizoctonia* and Early Blight were prevalent in the LaCrosse section in certain years. Such diseases are very disturbing in time-of-planting tests, since blight epidemics spread readily from older to younger plantings in adjoining plots in spite of vigorous control measures. Notations were made of the time of initial infection, the progressive spread and the relative damage to foliage of the potatoes of each planting, and these factors were given due consideration in the final interpretation of results, but no correction of data was attempted.

Continuous thermographic soil temperature records were made during the growing season at the depth of the seed-piece (approximately 2.5 inches deep at Hastings and somewhat deeper at LaCrosse). Soil moisture determinations were made at weekly intervals during the growing period from each plot by taking composite samples with a soil tube to a depth of 1 foot, measured from the top of the ridge. The percentage of soil moisture was computed on an oven-dry basis.

The time of harvesting each planting was determined, to some extent, by the weather and by the farmer-cooperator. In general, from 90 to 100 days were allowed from planting date to harvest. Individual hill records were taken of 75 to 150 hills from a number of replications. There were 8–12 replications per planting. These records included total number and weight of tubers per hill, number of 1's and 2's, and their respective weights. The remaining hills of each replication were combined so that total weights and total number of tubers were secured for each replication. The yield in barrels[2] per acre of United States grade no. 1's, or prime tubers, is used in this study as a measure of the most effective time of planting. The relative yield of prime potatoes per acre is governed by the number of such tubers per hill and by their size or weight. Some consideration has also been given to the yield of no. 2's, even though any planting having a large proportion of this grade of tubers may be regarded as having been produced under unfavorable environmental conditions.

Weights of tubers were recorded to the nearest gram, using a wire basket as a container, which facilitated shaking out any loose dirt prior to weighing. In most cases the tubers from the sandy soil were relatively clean when dug, although it was necessary to wash some of them.

In converting the yield in grams per plot to barrels per acre, a constant number of hills per acre, 10,700, was assumed, based upon rows 40 inches apart and hills spaced 14.5 inches in the row. Then by the use of a constant derived from the fraction in the formula, $Y \times \frac{1}{74{,}844.A}$ barrels per acre, comparable results throughout the tests were assured. In this for-

[2] A barrel contains 11 pecks or 165 pounds of potatoes.

TABLE I

AVERAGE YIELDS PER ACRE OF PRIME POTATOES AT LACROSSE AND HASTINGS, FROM 1926 TO 1931

YEAR AND PLACE	DATE OF PLANTING								ANNUAL MEAN
	DEC. 15	JAN. 1	JAN. 15	JAN. 21	JAN. 28	FEB. 4	FEB. 11	FEB. 20	
	bbl.	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	*bbl.*	
LaCrosse 1926–27	.....	.....	35.01	33.80	8.20	34.00	46.10	51.00	34.69
LaCrosse 1927–28	.....	.....	60.05	73.35	60.60	69.75	64.15	52.30	63.37
Hastings 1927–28	.....	.....	.....	46.00	47.95	52.58	46.88	37.30	46.14
1928–29	32.86	20.01	34.94	40.26	13.18	5.47	3.67	.....	21.48
1929–30	30.19	23.67	18.69	26.63	19.35	13.99	7.16	8.19	18.48
1930–31	16.76	40.38	39.13	26.17	28.15	17.32	10.74	5.83	23.06
Mean (Hastings only)	26.60	28.02	30.92	34.77	27.16	22.34	17.11	17.11	.....

* Average probable error for all plantings = ± 0.74 barrel per acre.

mula Y represents the yield in grams per plot of area A, while 74,844 is the number of grams in a barrel of potatoes weighing 165 pounds net.

Experimental results

I. Yields of no. 1's and no. 2's

The yields of United States grade no. 1 potatoes in barrels per acre are shown in table I for the various plantings at LaCrosse and Hastings during the 5-year period from 1927 to 1931 inclusive.

Table I shows two facts of particular interest: (1) The wide variation in yield of no. 1's secured from plantings on the same date[3] but in different years; and (2) the uniformity of the mean yield over a considerable part of the planting season tested. Maximum yields were not secured twice for any one planting date. In 1928, the February 4 planting was best, while the largest yields came from the January 21, December 15, and January 1 plantings in the successive years. Furthermore, the growing conditions in 1928 were favorable throughout the whole planting range[4] as compared with

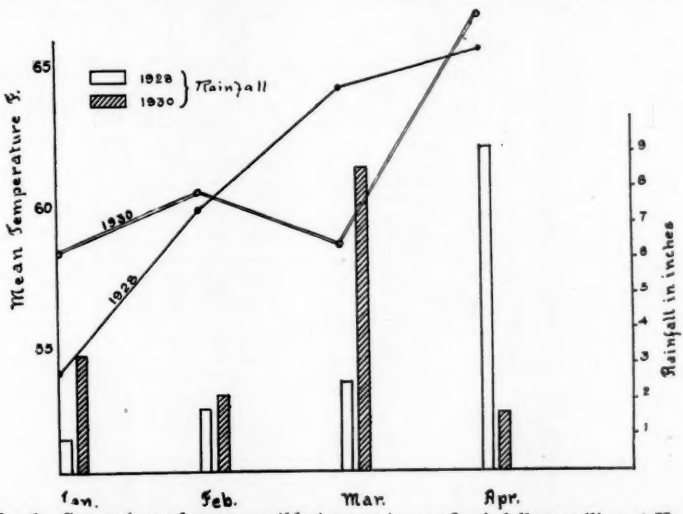


FIG. 1. Comparison of mean monthly temperature and rainfall prevailing at Hastings during the growing seasons of 1927–28 and 1929–30. The former was characterized by large and the latter by small yields. The weather conditions obtaining during February of these years were not essentially different, but the wide divergences for the other months, especially that of March, should be noted.

[3] It was impractical to plant on exactly the same date each year but the variation was not more than three days, so that the nearest average date of planting is shown in the tables and graphs.

[4] Early commercial plantings in the same fields with the experimental plots produced good yields.

TABLE II

PROPORTION OF PRIME, NO. 2'S, AND CULL POTATOES BY WEIGHT, FROM LACROSSE AND HASTINGS, 1927–28 TO 1930–31

YEAR AND PLACE	DATE OF PLANTING											
	DEC. 15			JAN. 1			JAN. 15			JAN. 21		
	1's	2's	CULLS	1's	2's	CULLS	1's	2's	CULLS	1's	2's	CULLS
	%	%	%	%	%	%	%	%	%	%	%	%
LaCrosse												
1927–28	.....	.....	.....	.....	.....	.....	70.4	19.6	10.0	80.4	13.2	6.4
Hastings												
1927–28	.....	.....	.....	.....	.....	.....	.....	.....	.....	68.7	23.1	8.2
1928–29	58.8	28.2	13.0	47.5	41.2	11.3	61.5	27.1	11.4	61.8	31.8	6.4
1929–30	53.6	24.2	22.1	52.3	23.8	23.9	44.0	30.0	26.0	49.8	26.7	23.5
1930–31	40.6	29.1	30.3	59.2	24.3	16.5	63.3	22.7	14.0	45.9	30.5	23.6
Mean	51.0	27.2	21.8	53.0	29.8	17.2	59.8	24.8	15.3	61.3	25.1	13.6
Mean for Hastings only	51.0	27.2	21.8	53.0	29.8	17.2	56.2	26.6	17.1	56.5	28.0	15.4

TABLE II—(*Continued*)

Year and place	Date of planting											
	Jan. 28			Feb. 4			Feb. 11			Feb. 20		
	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls	1's	2's	Culls
	%	%	%	%	%	%	%	%	%	%	%	%
LaCrosse												
1927–28	76.5	15.4	8.1	79.0	12.9	8.1	75.5	15.0	9.5	67.6	18.2	14.2
Hastings												
1927–28	62.8	19.4	17.8	72.6	20.6	6.8	63.8	26.1	10.1	59.7	28.6	11.7
1928–29	32.9	44.9	22.2	16.3	52.1	31.6	11.8	46.9	41.3	.....	.....	.....
1929–30	38.2	31.5	30.3	37.3	28.3	34.4	25.9	29.2	44.9	30.0	31.9	38.1
1930–31	53.3	32.1	14.6	42.6	32.7	24.7	37.3	32.3	30.4	31.8	33.9	34.3
Mean	52.7	28.6	18.6	49.6	29.3	21.1	42.9	29.9	27.2	47.5	28.2	24.6
Mean for Hastings only	46.8	31.9	21.2	42.2	33.4	24.4	34.7	33.6	31.7	40.5	31.5	28.0

those prevailing in 1929–30. These facts indicate that certain climatic factors in specific years are not only more conducive to high yields (fig. 1), but that at certain times during the normal growing period of each year there are more favorable relations than at others. The relatively dry cold January and the dry warm March of 1928 seem to be significant as related to high yields of prime tubers. A comparison of other high and low yielding years confirms this relationship.

The production of a large percentage of no. 2 potatoes is undesirable since strong competition on the markets has made it increasingly difficult to sell this grade of tubers advantageously. For this reason the yield data contained in table II are interesting.

Table II shows that one-fourth to one-third of the total weight of tubers produced during the 5-year period were no. 2's, and that as a rule the larger proportion of this grade was secured from the latest plantings. This was especially noticeable in the last three plantings in the season of 1928–29. There are two explanations for this condition: (1) Late Blight often killed immature vines, thus arresting the growth of tubers that would otherwise have become no. 1's; and (2) the later plantings were harvested from 78 to 90 days after planting in contrast to 100 to 110 days for the earlier plantings. The large proportion of culls (no. 3's and 4's) in many of the plantings should also be noted, for these represent waste as a general rule. The highest percentage of no. 1's, as well as the largest yields, were secured on the average from the January 15 and January 21 plantings.

II. Effects of rainfall and soil moisture on yield and grade of tubers

A mathematical study of the relationship between yield of no. 1 tubers per acre[5] and rainfall by months during the potato growing season at Hastings for a 10-year period shows that relatively dry Marches and wet Aprils are conducive to high yields.[6] The experimental plantings covering the 5-year period also confirm this fact.

To determine the relative importance of soil moisture at any particular stage of potato development upon the yield of no. 1 tubers, four stages of growth were studied as follows: (1) the period of germination, *i.e.*, from the date of planting until a majority of the sprouts were above ground,—usually from 20 to 24 days after planting; (2) the period of stolon formation, which usually begins in the faster growing hills about the 28th day

[5] Average yield data for the entire area at Hastings were furnished through the courtesy of the Hastings Potato Association.

[6] The following correlation coefficients were derived:

December 0.091 ± 0.18; January -0.231 ± 0.20; February 0.063 ± 0.16;

March -0.77 ± 0.087; April 0.65 ± 0.12; entire growing period -0.12 ± 0.20.

It was also shown that wet Marches have a tendency to be cold, the correlation coefficient being -0.40 ± 0.07.

after planting and continues through the 7th week or 50th day in the slower-growing plants; (3) the tuber-setting and tuber-growing period, which begins immediately after stolon formation and extends until harvest; (4) the entire growing period from planting until harvest.

Repeated observations in the field showed that the interval required for stolon formation of any one plant is from 7 to 10 days, and in any planting of 200 or more hills the process will reach its maximum about 35 to 40 days after planting, depending upon the growing conditions. Different varieties show some variation in the time of stolon formation; the data given are for the Spaulding Rose variety.

The partial correlation studies noted above indicated a distinct relationship between the yield of no. 1 tubers and the percentage of soil moisture during the period of stolon formation (28–50 days after planting). No well defined relationship appeared for the other growth periods noted. The yield-moisture relationship is shown in figures 2 and 3 for LaCrosse and Hastings respectively, for the season of 1927–28. The average soil moisture for the 28th to 50th days after planting was used as a basis of comparison with yields from the respective plots. In figure 2 the average soil moisture and the respective yields were plotted for each plot in all the plantings, while in figure 3 only the averages of all the plots for each planting were plotted against the average yield for that planting.

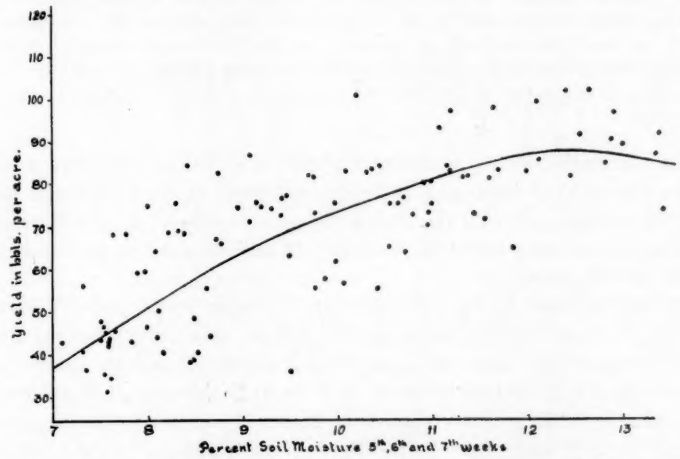


FIG. 2. Correlation between average soil moisture content during the stolon formation period and yield of no. 1 tubers in barrels per acre at LaCrosse, 1928. The curve was derived from a treatment of the data by the method of least squares. Note tendency toward an inverse relationship as the soil moisture rises above 12 per cent.

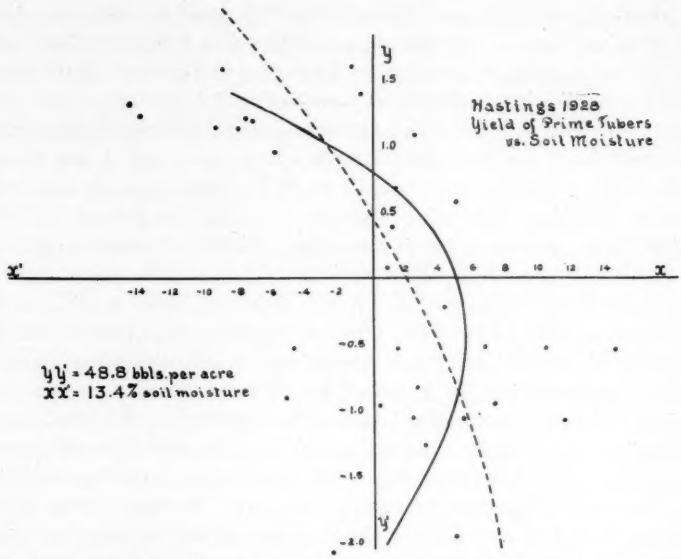


FIG. 3. Correlation between average soil moisture during the stolon formation period and yield of no. 1 tubers in barrels per acre at Hastings, 1928. Dotted curve was derived from a treatment of the data by the method of least squares, while the solid curve represents an interpretation of the data based upon field notes. The latter, likewise, more nearly fits the facts as derived from the other years of the investigation, especially with respect to the point of optimum moisture (about 12.5 per cent.). This is in reality a continuation of the curve shown in figure 2, showing inverse moisture-yield relationship.

The correlation shown in figure 2 (0.806 ± 0.023) is large and positive; that is, the yield of tubers at LaCrosse *increased* as the percentage of soil moisture *increased* during the stolon formation period. As the percentage of soil moisture went above 12, however, there was a distinct tendency for a reduction in yield.

The soil moisture content is considerably higher in all plots at Hastings than at LaCrosse over the same period of time, as a comparison of figures 2 and 3 will show. The range at LaCrosse was from 7 to 13 per cent., while at Hastings it ranged from 11.5 to 15.3 per cent. Therefore the correlation (-0.743 ± 0.18) shown in figure 3 is seen to be negative in contrast to that shown in figure 2; *i.e.*, the yields of prime tubers at Hastings *decreased* as the soil moisture *increased* during the stolon formation period. The most favorable moisture, however, appears to lie near 12 per cent., the same as at LaCrosse. The variation of this apparent optimum from year to year was not marked. In 1928–29 the largest

yields were obtained from plots showing approximately 13 per cent. of moisture. There is some evidence, although it is not conclusive, to show that there is an interrelationship between the mean temperature during the stolon formation period and the moisture optimum. It is important to note that the peak of the stolon formation period is in March for the major plantings at Hastings and LaCrosse.

III. Effect of temperature on yield and grade of tubers

A comparison of the soil temperature with that of the air, the latter recorded by the United States Weather Bureau field station at Hastings, is shown in figure 4, covering the major portion of the potato growing

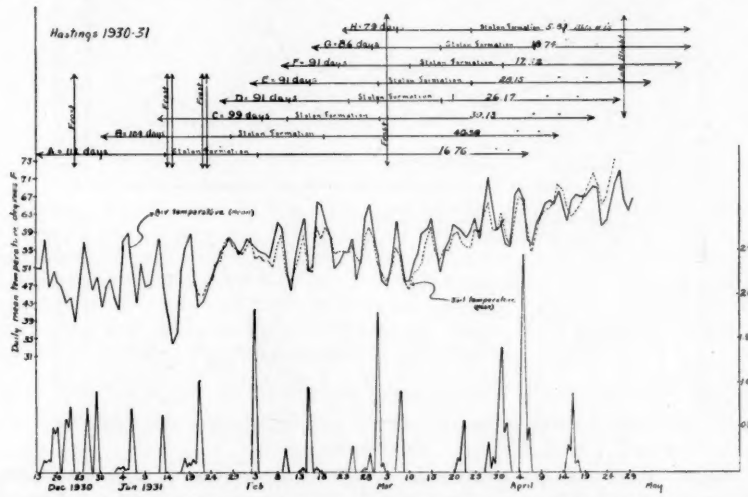


Fig. 4. Phenological relations prevailing during the potato growing season of 1930–1931 at Hastings, Florida. Number of days indicated for each planting represents time from planting to harvest. Note that frost occurred on six different days, damaging the plants of *A* severely during the stolon formation period, and those of *D* less seriously for the similar stage of growth, resulting in reduction in yield in both instances. The low yields for plantings *G* and *H* were primarily due to the short growing period subsequent to stolon formation, owing to blight incidence. Note that the soil temperature was lower during March and higher during April than corresponding temperature of the air.

season of 1930–31. The mean soil temperature for the period shown was 62.9° F., while the mean air temperature was 63.2° F. for the same period. It seems important to note that during March the mean soil temperature was nearly 3° below the air temperature, while at the end of the season during April the soil temperature rose above the air temperature, thus

making the two means for the period practically equal. Similar relations were found for the other years of this study.

In figures 5 and 6 are shown the average soil temperatures prevailing during the stolon formation period (28–50 days) for the successive plantings at LaCrosse during 1927–28 and for Hastings 1930–31, respectively, as they are related to yields and moisture, together with *Rhizoctonia* prevalence at LaCrosse. These examples are typical. The close coincidence of *Rhizoctonia* prevalence with changes in moisture is particularly interesting.

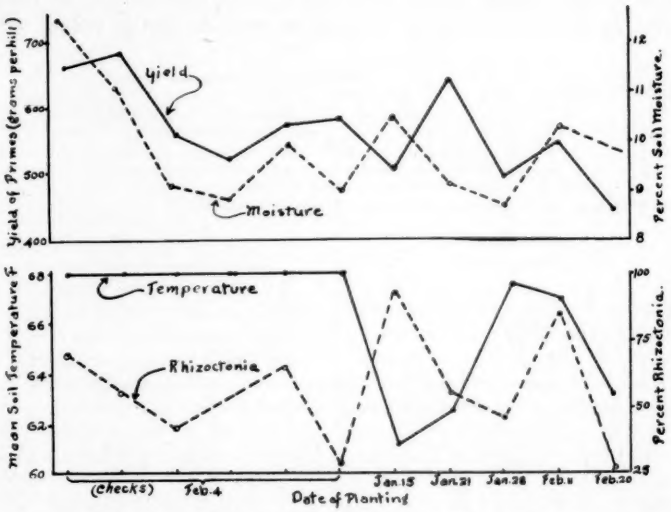


FIG. 5. When the temperature range is between 53° and 68° F. there seems to be little measurable effect upon potato yields. There is no apparent correlation between them. Both the moisture and the *Rhizoctonia* frequency curves, however, show direct correlation with yield. The one exception is noted with the January 15 planting where low temperature and high moisture favored the very high *Rhizoctonia* incidence (91 per cent.).

The experiments for the five years indicated that the mean temperature plays a relatively less important part in determining yields than does soil moisture. Only in two cases was the temperature so cold that germination and the initial growth were seriously retarded, namely, the January 15 planting of 1928 and the January 1 planting of 1931. There is some evidence in the correlation to show that the high temperatures during dry Aprils militate against high yields.

Temperature indirectly affects yields since diseases such as *Rhizoctonia* and Late Blight thrive best under relatively cool moist conditions, as shown

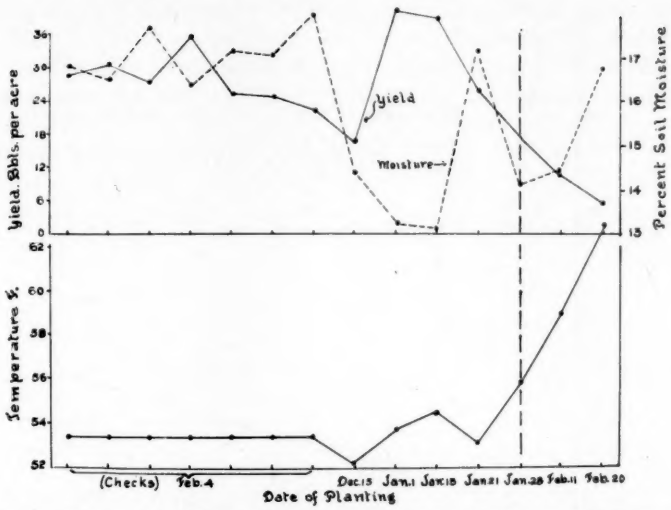


FIG. 6. Temperature effects are apparently of little significance during the period of stolon formation as judged by yield. Note that the checks (February 4 planting) were grown under the same temperature but under different moisture conditions and that the latter factor showed a decided inverse relationship to yield, with the possible exception of the December 15 and January 28 plantings. Mean temperature for the former period was unusually low, retarding growth visibly. Plantings from January 28 to February 20 were prematurely diseased by Late Blight.

by RICHARDS (**6**) and by our own experiments. The effects of freezing temperatures upon the growth and yield of potatoes are given in a separate publication. The chief conclusion was that the period of stolon formation (28th–50th day after planting) is a critical one, and that when vines are frozen to the ground at that stage the yields are reduced more than if they are frozen at any other time. It is important, therefore, to know the probabilities of the occurrence of freezing weather at various stages of potato growth in the Hastings and LaCrosse areas. Based upon Weather Bureau records of 40 years, the data shown in figures 7 and 8 are at least highly suggestive. The coldest weather usually comes during the first two decades of January and in the same intervals during February, as shown in figure 8. The time of stolon formation (critical period) occurs during the first half of February in plantings made prior to January 15–20. When the plantings are deferred to the last ten days of January, however, then the critical period falls during the milder weather which characterizes the latter part of February and the first days of March (fig. 7). This may be one reason why the late January plantings, over a long period of years, are said by growers to have yielded the largest crop of marketable tubers.

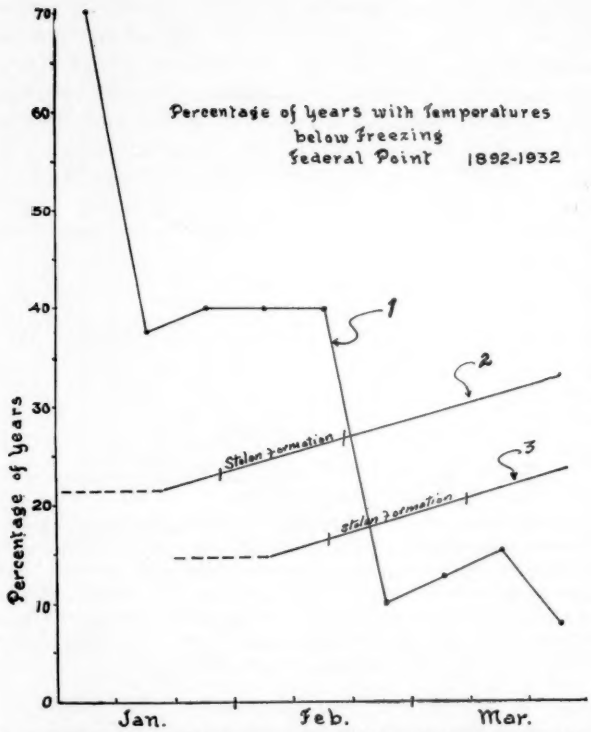


FIG. 7. Curve 1 shows freezing frequencies at 10-day intervals expressed in percentage of years during the months of January, February, and March. Two growth curves for potatoes are superimposed. Curve 2 represents the distribution of growth stages for plantings made about January 1. Curve 3 shows the distribution for plantings made about January 20. Dotted portion of curves 2 and 3 represents the time prior to appearance of sprouts above-ground. Note how stage II of curve 2 coincides with the high frequencies of cold weather the first two decades of February, while the same stage in curve 3 coincides with the milder weather the last of February and the first part of March.

When the mean monthly temperatures prevailing during January, February, March, and April, respectively, are compared with the yield of no. 1's over a 10-year period, some interesting relations are shown.[7] Cool Januaries and warm Marches seem to favor the growth and yield of no. 1 tubers (fig. 1). There is also an indication that cooler than normal

[7] The correlation coefficients for yields and mean temperature for the months of January, February, March, and April are -0.51 ± 0.15, 0.20 ± 0.20, 0.51 ± 0.16, and -0.20 ± 0.20 respectively.

weather in April increases yield. These facts suggest that our temperature curve in Florida is upside down as it relates to yields. As a matter of fact, it is almost precisely inverted as compared with the normal temperature curve for Maine as shown in figure 9. The average temperature

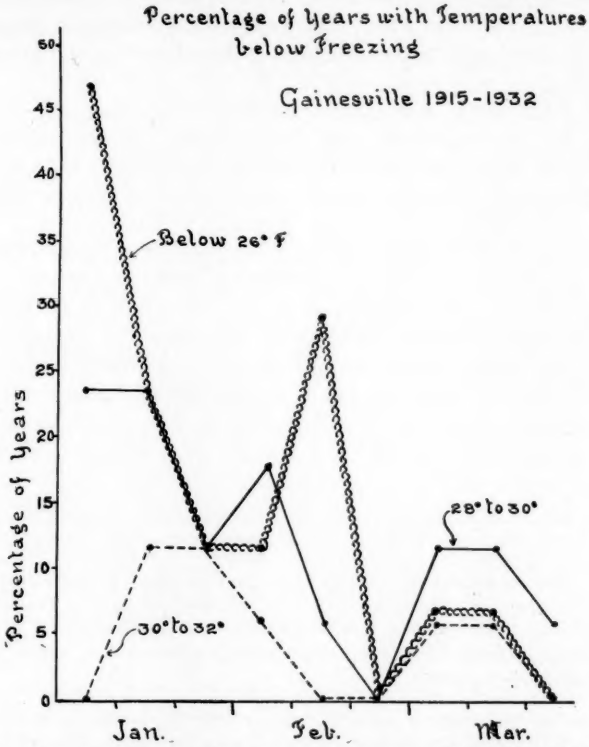


FIG. 8. Freezing frequencies for January, February, and March at 10-day intervals expressed in percentage of years. Subfreezing temperatures have been divided into three groups: 32°–30°, 28°–30°, and 26° and below. Note the high frequencies of the colder temperatures during the first part of January and the second 10-day period in February. The most frequent freezing temperatures for March are between 28° and 30°.

range during the last two months in Maine should be ideal for starch storage according to BUSHNELL'S findings (**1**), while in Florida the rate of respiration is too high.

Discussion

The data show potatoes to be very sensitive to soil moisture variation, especially during the period of stolon formation. These findings accord

well with those of WIDTSOE (**8, 9, 10**), HARRIS (**4, 5**), and SMITH (**7**). In an area like Hastings, where irrigation water is easily available, there should be no reason why potatoes need suffer for water during this period. In fact, the most usual problem would be to prevent too much soil moisture. This of course suggests the need for better drainage. The opposite conditions prevail at LaCrosse where somewhat different soil and subsoil conditions obtain and where better drainage is facilitated by the greater slope of the land.

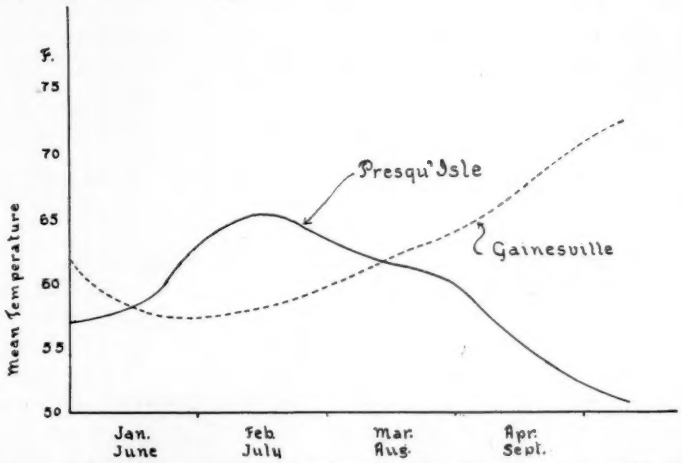


FIG. 9. Smoothed mean daily temperature data covering ten years (1917–1926 inclusive) for Presque Isle, Maine, and Gainesville, Florida, during their respective potato growing seasons. Note almost precise inversion of temperature curves. Correlations between yield and temperature at Hastings indicate that January and April are too warm while March is too cold.

There is an interesting question regarding the possible recurrence of high rainfall during March (period of stolon formation). If there are cycles, when do they come? In order to answer this question the rainfall record of March at Jacksonville since 1872 was submitted for harmonic analysis.[8] Among the cycles disclosed there were two of outstanding importance, approximately 10 and 7.5 years respectively. The reality and importance of these may be judged by the fact that the series of wet years in these cycles yielded an average of 199 and 223 per cent. more rainfall, respectively, than the corresponding series of dry Marches. It should be remembered that dry Marches were found to foster larger yields at Hastings.

[8] The analysis was made through the courtesy of DAYTON C. MILLER, Case School of Applied Science, Cleveland, Ohio.

The potato is apparently not so sensitive to the temperature variations that normally obtain in the Hastings area,—54° to 65° F. When mean monthly temperatures above or below these limits occur there appears a noticeable limitation of yield. The inversion of the temperature curve in Florida as compared with that in Maine may explain in part the lower yields that are common to Florida.

Summary

1. There is a considerable latitude admissible in the time of planting Irish potatoes in the LaCrosse and Hastings areas, ranging from mid-December to the middle of February. The two-week period extending from the middle of January to the first of February gave the largest average yields.

2. The period of stolon formation, extending from about the 28th to the 50th day after planting, appears to be a critical one in the development of the potato as measured by yield of primes. The peak of this critical period is reached about the 35th to the 40th day after planting.

3. The soil moisture content during the critical period seems to be the chief factor involved in determining yields, the optimum soil moisture content being about 12 to 13 per cent., based upon oven-dry weights.

4. In the LaCrosse areas the soil moisture rarely reaches the optimum and therefore too little moisture is the limiting factor in production. On the other hand, the soil moisture at Hastings is usually well above the optimum owing to a more impervious subsoil, so that too much moisture is the limiting factor in production there. Better drainage at Hastings and a need for irrigation at LaCrosse are indicated.

5. The month of March seems to be a critical one for the average planting, since it approximates the time of stolon formation. For Hastings a warm, relatively dry period extending from late February through March is favorable to large yields, and *vice versa.*

6. Temperature effects seem to be of lesser importance in their influence upon yields, although there is evidence to show that the temperatures prevailing during January and April are too high while March temperatures are too low for maximum yields.

7. March temperatures tend to be below normal when the precipitation is above normal and both of these factors are inimical to large yields.

8. There are four periods or cycles in March rainfall of approximately 30, 15, 10, and 7.5 years respectively. The latter two have recurred over the past 60 years so that the wet Marches show 199 and 233 per cent. increase of rainfall, respectively, over the corresponding dry years.

9. When potatoes are planted during the latter half of January the critical period of stolon formation follows the period of greatest freezing

frequency, and therefore reduces the chance of low yields due to freezing injury.

The writer is indebted to Dr. DAYTON C. MILLER of the Case School of Applied Science, Cleveland, Ohio, for the analysis of the rainfall data and to Mr. J. W. KITE of LaCrosse and Mrs. GEORGE V. LEONARD of Hastings for the courteous and able assistance given in the cultural practices and for the use of the land upon which the tests were conducted.

FLORIDA AGRICULTURAL EXPERIMENT STATION
GAINESVILLE, FLORIDA

LITERATURE CITED

1. BUSHNELL, JOHN. The relation of temperature to growth and respiration in the potato plant. Minnesota Agr. Exp. Sta. Tech. Bull. 34. 1925.
2. ENSIGN, M. R. Some climatic factors as they affect potato production in Florida. Potato Assn. Amer. Proc. 1932.
3. HARDENBURG, E. V. A study, by the crop survey method, of factors influencing the yield of potatoes. Cornell Agr. Exp. Sta. Mem. 57. 1922.
4. HARRIS, F. S. The irrigation of potatoes. Utah Agr. Exp. Sta. Bull. 157. 1917.
5. ————, and PITTMAN, D. W. Irrigation experiments with potatoes. Utah Agr. Exp. Sta. Bull. 187. 1923.
6. RICHARDS, B. L. Further studies on the pathogenicity of *Corticium vagum* on the potato affected by soil temperature. Jour. Agr. Res. **23**: 761–770. 1923.
7. SMITH, J. W. The effect of weather upon the yield of potatoes. Weather Rev. **43**: 222–236. 1915.
8. WIDTSOE, J. A. The production of dry matter with different quantities of irrigation water. Utah Agr. Exp. Sta. Bull. 116. 1912.
9. ————, *et al.* Irrigation investigations in 1901. Utah Agr. Exp. Sta. Bull. 80. 1902.
10. ————, and MCLAUGHLIN, W. W. The right way to irrigate. Utah Agr. Exp. Sta. Bull. 86. pp. 53–101. 1903.

STOCK–SCION CHEMISTRY AND THE FRUITING RELATIONSHIPS IN APPLE TREES

HAROLD L. COLBY

Introduction

The Malling Nine or "true Paradise" apple stock has been vegetatively propagated for centuries as a rootstock for use in the production of dwarf apple trees. Malling Nine is said to be native to parts of southern Russia (**3**), and is presumably a type that has adapted itself to semi-arid conditions. The stock itself is a low branching shrub, rather than a tree. As to the physiological effects in dwarfing the scions (**11**) of apple varieties grafted or budded upon it, very little is known. BLACKMAN (**1**) states: "The influence of stock on scion is one which has puzzled plant physiologists for decades. No progress has been made as to the mechanism of the action of a dwarfing stock."

The Malling Twelve stock used in this work is one of extreme vigor among the vegetatively propagated stocks (**8**), closely approaching the vigor of the free-growing or seedling crab stocks in common use by American nurserymen for the production of standard budded or grafted apple trees. The two stocks, Malling Nine and Malling Twelve, represent very nearly the extremes in growth habit, vigor, age of fruiting, etc., found in the *Pyrus malus* species. Of the scion varieties used, Whitney is a very low N tree, slowing up in growth at an early age, while Snow is an extremely high N variety, very vigorous in growth.

A chemical study of the trees grown on the two stocks just mentioned should reveal something of the chemistry involved in growth extremes in apple, and possibly something of the fruiting relationships as they occur in dwarf and in standard apple trees of different varieties.

All apple scion varieties are not affected in the same way by these two Malling stocks, according to ROBERTS'S (**14**) work. Of the varieties studied here, Wealthy is only moderately dwarfed by the Malling Nine stock, but is fruiting heavily (at the age of 6 years) on a biennial schedule. McIntosh on Nine is decidedly dwarfed, with characteristically open top, and fruits heavily. Spy, Winesap, and Whitney are severely dwarfed, and are fruitful. All of these varieties as grown on Malling Twelve stock are as yet entirely unfruitful (with the exception of Wealthy which fruits lightly) and are very vigorous growers.

Snow on Nine is not appreciably dwarfed, however, while Snow on Twelve makes a fair growth but does not show the extreme vigor that might be expected from this combination of root and scion. York in

its early years does poorly on Malling Twelve, and grows better on Malling Nine. Both Snow and York are extremely high N trees, the highest encountered in this work.

In the future, some very important uses of certain clonal rootstocks, or of double-working on known varieties which scion root readily, seem to be to improve the hardiness of certain apple varieties in the northern states, and to improve N nutrition in early spring, effecting a better set (**13**) of fruit on some high-temperature varieties planted in northern states. Years ago it was reported in the north that some of the southern plums (Wild Goose) bore fruit well when the tree was double worked on the trunks of certain northern plum varieties. On seedling roots Wild Goose usually bloomed but failed to set fruit. Pollinization was not always lacking. Nutritional stock-scion effects seem dominant in such cases. The vigorous hardy Virginia crab when double-worked may play a similar rôle with some apple scion varieties that set fruit poorly in the north.

Experimentation

Most of the results recorded here were obtained from 6-year old trees dug in July, 1933, from the University orchards at Madison, Wisconsin. A few more trees were dug in October, 1933, and also in June, 1934. The material was available through the courtesy of Dr. R. H. Roberts of the University of Wisconsin. The weight yields and trunk circumferences of the vigorous groups of trees at the time of harvest are given in table I. The top-root weight ratios are interesting, Whitney on Nine and Snow on Nine both showing slightly higher top-root ratios than the same scion varieties on Malling Twelve stocks. Whitney on seedling root showed a low top-root ratio as compared with Snow on seedling, or with Winesap or Spy on seedling.

Seasonal shoot and root growth

Whitney on Nine and Whitney on Twelve shoots grow at about the same rates from early spring to May 26 (**18**), after which date Whitney on Twelve shoots grew rapidly, while Whitney on Nine shoots soon stopped terminal growth almost entirely. Spy on Nine shoots are slower in growth than Spy on Twelve shoots from the very start of the buds in early spring. McIntosh on Nine, with rather few terminals, grows much faster in early summer than does McIntosh on Twelve, but the former slows down shoot growth and stops in July, while McIntosh on Twelve shoots are growing rapidly. The Malling Twelve stock itself at Madison is very late in the spring in bursting its buds (April 30), and the shoots grow slowly until about June 1, then start in rapid growth. Its roots are slow in starting terminal growth in the spring, but were growing more rapidly by the

TABLE I

WEIGHT YIELDS PER TREE AT TIME OF HARVEST

Scion Stock	Dry weight of young roots	Dry weight of root trunk	Air-dry weight of top	Trunk circumference
Series I: July	*gm.*	*gm.*	*gm.*	*cm.*
Mailing 9*	22.4	35.0	101.0	.....
Mailing 12*	27.2	20.0	176.0	5.1
Whitney on 9	65.0	40.0	248.0	5.4
Whitney on 12	690.0	372.0	2,255.0	12.8
Whitney D. W.† on 9	219.0	58.0	852.0	8.0
Whitney D. W. on 12	652.0	433.0	2,310.0	11.9
Whitney on Snow on 9	280.0	75.0	1,170.0	8.7
Whitney on Snow on 12	290.0	73.0	1,210.0	8.7
Series II: July				
Whitney on seedling	1,626.0	231.0	10,300.0	19.7
Snow on 9	1,019.0	374.0	9,900.0	21.0
Snow on 12	947.0	662.0	11,000.0	21.2
Snow on seedling	1,092.0	620.0	14,960.0	23.7
McIntosh on 9	468.0	265.0	6,200.0	16.2
McIntosh on 12	1,100.0	602.0	12,000.0	22.8
Baldwin on Spy	325.0	302.0	3,000.0	15.2
Baldwin on Winesap	1,000.0	382.0	9.950.0	23.6

* Cut back for scion wood; not comparable with other trees.

† Double-worked; *i.e.*, seedling roots, with Malling 9 interpiece, etc.

end of May. This type of behavior seems rather common in trees that continue growth late into summer and fall. Malling Nine shoots started growth April 18, and the root tips (new growth) were one-half inch long by April 20. The small roots back of the tips did not seem to be heavily suberized, and perhaps were not entirely dormant during the winter months. Malling Twelve roots were dormant until April 30, with a well suberized root system.

Malling Nine rootlets soon suberize as the top foot of soil dries out; later cycles of root tip growth occur after heavy summer rain storms.

Working with McIntosh trees in British Columbia, Rogers (**16**) found that as the soil dried out in mid June the first major cycle of new root length growth declined rapidly, and at the same time terminal shoot growth slowed down and soon stopped. After the soil moisture was replenished by irrigation, new root tip growth again occurred, but without breaking the dormancy of the shoot terminal buds. New root tip growth suberizes quickly when soil moisture becomes low, and terminal shoot growth seems to stop at the same time, although the leaves do not wilt. It was shown by Harvey (**7**) that the terminal shoot growth of Grimes

trees stopped before the water content of the top one-third of the shoot growth fell to 63 per cent. A water content of 70 per cent. allowed rapid shoot growth. Apparently a *suberized* root system can usually supply water fast enough to keep the leaves from wilting, but does not furnish the large water supply or "turgor" needed for sustained apical shoot growth.

In harvesting the Malling Nine stock it was noted that the bark thickness of root and stem was far greater than that of the Malling Twelve stock. A similar situation exists in the bark-wood ratio of the shoots of Whitney on Nine compared with Whitney on Twelve.

EFFECTS OF STOCK ON LEAF AREA

SCION	STOCK	DATE	COMPARATIVE SIZE PER LEAF	
			SPUR LEAVES	SHOOT LEAVES
Whitney	Nine	May 26	58.5	100.0
Whitney	Twelve	May 26	31.6	100.5

McIntosh spur leaves from trees grown on the two stocks showed a similar situation as to the extent of average leaf surface. Also there are fewer leaves per spur on trees of these varieties grown on Malling Twelve than when grown on Malling Nine stock.

NITROGEN CONTENT OF TREE TISSUES

In table II are presented the total N figures in percentage dry weight of roots, shoots, etc. The "young roots" portion refers to all roots exclusive of the root trunk. Where an intermediate piece of another variety was used in the stem, the bark and wood of the interpiece were analyzed separately. A section of the stem trunk 6 inches long just above the interpiece was taken for analysis also (July).

The root trunk and stem trunk parts of Malling Nine, or of Whitney on Malling Nine trees, were high in N while shoots of Malling Nine were low in N. There appears to be a striking lack of "polarity" in the N distribution in the Malling Nine tree itself (contrasted with that of Malling Twelve). The older portions of the Malling Nine stock were well supplied with N, also with P, fat, and starch while the shoot tips were low in N and P. Whitney double-worked with Malling Nine interpiece on seedling root showed that the dwarfing interpiece resulted in extremely high N in the young roots and root trunk; and that the interpiece itself was twice as high in N as the Malling Twelve piece of the Whitney double-worked with Malling Twelve inter on seedling stock. Starch and P showed a similar accumulation below and in the Malling Nine section.

TABLE II

TOTAL NITROGEN IN PERCENTAGE OF DRY WEIGHT

STOCK SCION	LEAVES		SHOOTS		STEM TRUNK (ENTIRE)	INTER-PIECE (ENTIRE)	ROOT TRUNK	YOUNG ROOTS	TREE WEIGHT
	TOP	BASE	TOP	BASE					
Series I: July	%	%	%	%	%	%	%	%	*gm.*
Malling 9	2.2	2.1	1.2	0.7	0.48		0.54	0.70	158
Malling 12	2.1	2.0	2.1	1.1	0.23		0.31	0.72	223
Whitney on 9	2.2	2.3	0.9	0.7	0.33		0.51	0.68	353
Whitney on 12	2.2	2.4	0.9	0.7	0.24		0.29	0.65	3,317
Whitney D. W. on 9	2.2	2.3	0.9	0.6	0.24	0.43	0.58	0.87	1,129
Whitney D. W. on 12	2.2	2.5	1.0	0.8	0.23	0.22	0.21	0.46	3,395
Whitney on Snow on 9	2.1	2.4	1.1	0.7	0.22	0.24	0.31	0.66	1,525
Whitney on Snow on 12	2.0	2.4	1.0	0.7	0.24	0.26	0.30	0.54	1,573
Series II: July					BARK				
Whitney on seedling	2.2	2.4	1.0	0.8	0.70		0.33	0.68	12,157
Snow on 9	2.6	2.4	1.9	1.1	0.75		0.27	0.60	11,293
Snow on 12	2.3	2.6	1.9	1.3	0.78		0.29	0.69	12,609
Snow on seedling	2.4	2.2	2.1	1.5	0.78		0.26	0.83	16,672
Spy on seedling	2.2	2.1	1.3	0.7	0.73		0.23	0.51	12,881
Baldwin on Spy	2.2	2.0	1.7	1.0	0.80		0.34	0.71	3,627
Baldwin on Winesap	2.2	2.3	1.7	1.0	0.93		0.24	0.50	11,432
Series III: October									
Whitney on seedling			1.0	0.6			0.35	0.68	14,066
Snow on seedling			1.2	0.9			0.33	0.83	21,710

The Whitney on Snow on Malling Nine root showed that the Snow interpiece had the opposite effect to that of the Malling Nine inter of the Whitney double-worked on Nine combination. The roots below the Snow interpiece were low in starch and N in July. But the Snow interpiece of Whitney on Snow on Twelve was not able to empty the Twelve root below it of starch, and the N content remained unaltered.

Snow grafted directly on Malling Nine also showed the ability nearly to empty the root of starch during the active growing season, and reduced the N content of the root to a comparatively low level. Snow on Twelve did not show these results.

As to the shoots, the N content seemed to be rather constant for a given variety in early July regardless of the rootstock used. The N content of young shoots is difficult to alter, although shoots of trees grown on Malling nine stock usually showed a slightly lower N content in early summer than did shoots of trees grown on Malling Twelve roots. The leaves of Whitney on Nine were 3 per cent. lower in water (July) than were the leaves of Whitney on Twelve. In all probability, if a lack of water supply or "turgor" pressure to the shoot tips develops during the course of elongation of the terminal bud, shoot growth *stops for the season in these dwarf trees.* We assume that the water and N supplying power of the Malling Nine root must be increased by the Snow scion. The movement of accumulated N reserves in the root of Malling Nine to the shoots in early summer seems to be stimulated by the Snow top. No scion roots were present on these trees.

The one and two year old branches of Whitney on Nine showed a high N and starch content, compared with similar tissues from Whitney on Twelve trees, at the time shoot growth stopped on the dwarfed trees, although the shoots of the two sets of trees showed only small differences in N content.

Phosphate is low in the shoot tips of Whitney on Nine (table III) shoots in July, but not in the shoot tips of Spy on Nine, which is also dwarfed in growth. In the clonal roots, the Fe and Mn contents tend to follow the N distribution.

Trees of most varieties double-worked with Wealthy interpiece made vigorous growth, and the shoot leaves were higher in N than the leaves of trees double-worked on Jonathan. The latter combination tended to dwarf tree growth, and resulted in shoots higher in Fe and Mn and lower in N than shoots of the trees double-worked on Wealthy (table IV).

Starch content of tree tissues

It is already known that starch occurs in plants in the form of three or four different isomers. The various glucosans in different plant species

TABLE III

PHOSPHATE IN PERCENTAGE OF DRY WEIGHT

SCION STOCK	SHOOTS		ROOT TRUNK	YOUNG ROOTS
	TOP	BASE		
Series I: July	%	%	%	%
Malling 9	0.16	0.29	0.52	0.57
Malling 12	0.21	0.17	0.16	0.29
Whitney on 9	0.27	0.38	0.36	0.66
Whitney on 12	0.38	0.33	0.18	0.39
Whitney D. W. on 9	0.28	0.45	0.58	0.71
Whitney D. W. on 12	0.38	0.35	0.16	0.32
Whitney on Snow on 9	0.38	0.34	0.29	0.56
Whitney on Snow on 12	0.37	0.28	0.23	0.30
Series II: July				
Whitney on seedling	0.39	0.34	0.31	0.69
Snow on 9	0.41	0.38	0.29	0.58
Snow on 12	0.40	0.35	0.26	0.39
Snow on seedling	0.42	0.33	0.35	0.70
Spy on seedling	0.40	0.40	0.24	0.44
Baldwin on Spy	0.43	0.30	0.27	0.48
Baldwin on Winesap	0.42	0.28	0.19	0.33
Series III: October				
Whitney on seedling	0.35	0.28	0.17	0.41
Snow on seedling	0.36	0.32	0.28	0.44
Wealthy on 9	0.37	0.36	0.26	0.73
Wealthy on 12	0.34	0.28	0.15	0.68

TABLE IV

TOTAL N IN PERCENTAGE OF DRY WEIGHT AND FE IN P.P.M. IN TISSUES OF DOUBLE-WORKED APPLE TREES

SCION VARIETY	LEAVES (JULY 31)				SHOOTS (AUGUST 31)			
	ON WEALTHY		ON JONATHAN		ON WEALTHY		ON JONATHAN	
	N	FE	N	FE	N	FE	N	FE
	%	*p.p.m.*	%	*p.p.m.*	%	*p.p.m.*	%	*p.p.m.*
McIntosh	2.20	92.0	1.92	92.0	0.95	40.0	0.74	44.0
Winesap	2.15	100.0	2.13	112.0	0.93	42.0	0.77	60.0
Wealthy	2.55	98.0	2.31	108.0	0.91	42.0	0.79	55.0
Dutchess	2.77	92.0	2.42	100.0	0.90	47.0	0.86	58.0
Whitney	2.59	90.0	2.43	96.0	0.88	51.0	0.88	61.0
Spy	2.68	94.0	2.18	107.0	0.89	38.0	0.95	45.0
Snow	2.34	106.0	2.39	111.0	1.04	38.0	1.27	44.0

are little understood, and analytical methods used in the past have been misleading in their results. In table V are given starch analyses of some of the stock-scion material by various old and new methods for purposes of comparison. In tables VI and VII are given the entire set of starch analyses by a new method developed by the writer at Wisconsin, which is considered the most convenient, satisfactory method available. Some of

TABLE V

STARCH IN PERCENTAGE DRY WEIGHT BY VARIOUS METHODS

VARIETY OF GRAFT	DATE	NEW WISCONSIN METHOD	TWENTY PERCENT. ALCOHOL EXTRACT*	TAKA-DIASTASE	OFFICIAL ACID HYDROLYSIS
Shoots, top half		%	%	%	%
Whitney on Malling 12	July 14	0.25	0.10	0.60	17.1
Whitney on seedling	"	0.50	1.25	0.50	.....
Snow on seedling	"	0.10	0.10	0.70	.....
Snow on Malling 9	"	0.20	0.60	0.60	.....
Spy on seedling	"	0.15	0.80	0.80	.....
Winesap on Spy	"	0.15	0.80	0.80	.....
Baldwin on Spy	"	0.20	1.30	0.70	.....
Whitney on Malling 9	"	.....	.....	.....	.....
Bark of whole shoots	"	1.90	1.90	1.60	.....
Wood of whole shoots	"	0.60	0.60	0.60	.....
Whitney on Malling 12	"	.....	.....	.....	.....
Bark of whole shoots	"	0.66	0.35	0.35	.....
Wood of whole shoots	"	0.46	0.23	0.70	.....
Shoots, base half					
Whitney on seedling	"	1.00	1.40	0.70	.....
Snow on seedling	"	0.23	0.23	0.23	.....
Root trunk					
Snow on Malling 9	July 18	1.20	0.35	1.30	.....
Snow on Malling 12	"	0.93	1.20	2.10	.....
Young roots					
Malling 9	July 15	5.30	5.60	5.30	.....
Malling 12	"	1.60	2.80	2.50	.....
Shoots, top half					
Whitney on seedling	Oct. 10	4.00	4.00	3.90	.....
Snow on seedling	"	2.30	2.40	2.80	15.8

* Extract of tissue cleared with "nitric acid alcohol."

these samples were checked by the calcium chloride iodide method of starch analysis as proposed by DENNY (**4**). Taka-diastase holds no position of vantage, since ERLICH's (**5**) work on taka-diastase and the pectin compounds, and DENNY's suggestion that inulin or other allied substances are attacked by taka-diastase.

The starch method used in this work is a combination of several recently developed methods [DENNY'S, SULLIVAN'S (**18**) modification of the same, and NIEMANN'S (**12**)]. The fat free tissue was cleared with hot 85 per cent. alcohol plus 1 per cent. nitric acid for 30 minutes, the starch was extracted with hot 20 per cent. alcohol, then precipitated with IKI, using ammonium sulphate as the flocculating agent. The precipitate was washed with hot alcohol, taken up in hot water, and the iodine evaporated off; the starch was hydrolyzed in 2.5 per cent. HCl for 3 hours. The sugar determination was by gravimetric Munson-Walker. This starch method eliminates the use of calcium chloride as an extractive, since the latter is disagreeable to work with, slow in dissolving starch, and difficult to filter or handle otherwise because of its high viscosity. Ammonium sulphate is an excellent flocculating agent for starch iodide. If the presence of amylo dextrin in the sample is suspected, the precipitate should be washed with one-half saturated ammonium sulphate solution (**17**).

HOOKER'S (**10**) analyses for starch in apple tissues are among the best of the older analyses, but his results seem to have been badly in error, yielding too high figures in some early season shoot and spur analyses. He reported starch in Grimes shoots of May 21 as equal to 2.09 per cent., with a fall in starch to 1.4 per cent. by September 4. Many of the "starch" figures recorded in pomological literature are questionable or impossible.

Apple spur tissues taken later in the season give quite reliable starch data with either taka-diastase or the iodine methods. Certain varieties may be exceptions. The writer's observations showed that for work with low starch, high N succulent shoots, the taka-diastase (or malt diastase) method is worthless in starch determination and even gives "starch" figures showing trends opposite to the true relationships. Clearing with lead acetate does not give reliable starch results with some apple varieties. *The fruiting relationships tied up with the starch-N ratio cannot be studied accurately in apple when the taka-diastase method is used for the starch determinations.*

The starch analyses of the roots of dwarf trees showed that the usual assumption of "root starvation" in dwarf trees is erroneous. The dwarf roots were well supplied with starch, with fat, and with N compounds. The graft union of these trees was smooth and presented no serious interference to the downward movement of organic materials through the bark to the root. On the other hand, upward movement of organic reserves to the shoots of dwarf trees in early summer through the xylem and phloem may be interfered with by the low water supply moving through the root into the scion. The trachea and phloem cells of the Malling Nine roots are usually very small in diameter. The most outstanding anatomical feature of the "dwarfing" roots as altered to support vigorous top growth

TABLE VI

STARCH IN PERCENTAGE OF DRY WEIGHT

STOCK SCION	SHOOTS		STEM TRUNK		INTERPIECE		ROOT TRUNK	YOUNG ROOTS	TREE WEIGHT
	TOP	BASE	BARK (ENTIRE)	WOOD (ENTIRE)	BARK (ENTIRE)	WOOD (ENTIRE)			
Series I: July	%	%	%		%	%	%	%	*gm.*
Malling 9	1.30	2.50	3.0		.....	.....	4.1	5.3	158
Malling 12	0.10	0.30	0.4		.....	.....	1.7	1.6	223
Whitney on 9	0.80	1.40	1.4		.....	.....	3.7	4.4	353
Whitney on 12	0.25	0.55	0.22		.....	.....	1.6	3.7	3,317
Whitney D. W. on 9	0.80	1.50	1.5	1.5	2.1		5.1	5.6	1,129
Whitney D. W. on 12	0.70	1.30	0.30	0.35	0.26		2.1	3.7	3,395
Whitney on Snow on 9	0.40	0.75	0.70	0.25	0.70	0.50	1.3	2.5	1,525
Whitney on Snow on 12	0.40	0.75	0.85	0.20	0.65	0.40	2.3	3.9	1,573
Series II: July									
Whitney on seedling	0.50	1.00	0.25	0.20	.....	.....	1.1	3.6	12,157
Snow on 9	0.20	0.40	0.18	0.15	.....	.....	1.2	2.4	11,293
Snow on 12	0.20	0.40	0.35	0.35	.....	.....	0.9	3.6	12,609
Snow on seedling	0.10	0.23	0.20	0.20	.....	.....	1.5	2.9	16,672
Spy on seedling	0.15	0.30	0.45	0.30	.....	.....	1.5	4.3	12,881
Baldwin on Spy	0.20	0.40	0.50	0.80	0.60	0.35	1.6	2.4	3,627
Baldwin on Winesap	0.20	0.40	0.25	0.20	0.90	0.40	1.4	2.9	11,432
Winesap on Spy	0.15	0.30	0.30	0.25	0.20	0.35	1.7	3.5	11,077
Series III: October									
Wealthy on 9	3.3	.....	.....	.....	.....	.....	3.1	10.4	2,624
Wealthy on 12	3.0	.....	.....	.....	.....	.....	3.0	10.9	10.364
Whitney on seedling	4.0	.....	.....	.....	.....	.....	3.7	7.2	14,066
Snow on seedling	2.3	.....	.....	.....	.....	.....	2.3	4.4	21,710
Whitney D. W. on 9	4.3	.....	.....	.....	.....	.....	8.0	11.0	960
Whitney D. W. on 12	4.2	.....	.....	.....	.....	.....	7.7	10.8	3,200

TABLE VII

STARCH ANALYSES IN PERCENTAGE DRY WEIGHT OF FRUITFUL AND VEGETATIVE TYPES OF APPLE TREE TISSUE AT VARIOUS SEASONS OF THE YEAR

SCION ROOT	DATE	LOCATION	N	STARCH
			%	%
Spy on Malling 9				
Top half of shoots	July 20		1.24	0.3
Spy on Malling 12				
Top half of shoots	July 20		1.05	0.1
Spy on Malling 9				
Top half of shoots	Oct. 10		0.97	1.6
Spy on Malling 12				
Top half of shoots	Oct. 10		0.74	2.0
Spy on seedling				
Top half of shoots	July 20	Inner shady parts of tree	1.28	0.2
Spy on seedling				
Top half of shoots	July 20	Outer parts of tree	1.07	0.2
McIntosh on Malling 9				
Fruitful spurs (1-yr.) minus terminal bud	April 15		1.36	0.56
Terminal bud alone	" 15		2.00	0.01
McIntosh on Malling 12				
Spurs (veg. 1-yr.) minus terminal bud	April 15		1.06	1.15
Terminal bud alone	" 15		1.39	0.01
Whitney on Malling 9				
Two-year branches	June 13		0.55	0.73
Whitney on Malling 12				
Two-year branches	June 13		0.33	0.10
Whitney on Malling 9				
Top half of shoots	June 13		1.05	0.70
Whitney on Malling 12				
Top half of shoots	June 13		1.08	0.10
Gravenstein on seedling				
Two-year fruitful branches	June 16	Inner parts of tree	0.46	1.10
Gravenstein on seedling				
Two-year vegetative branches	June 16	Mostly outer parts of tree	0.33	0.70

of the Snow scion was the enormous development of large open vessels in the xylem of the Malling Nine young roots. This suggests that the water supply to the top was a dominant feature, affecting the rapid upward movement of starch, N, and P hydrolysis products from the root to the top. Trunk ring bark (**15**) graft dwarfing effects show that faulty xylem unions cannot be the sole cause of the dwarfing of apple scions (**20**). The Malling Nine phloem tubes and unions allow good *downward* transport to the dwarf

roots. *Upward* movement of foods in the spring through the Malling Nine phloem tubes may be very slow.

GRUBB (**6**) found that the amount of the effect of the interpiece on top growth of his trees was proportional to the length of the interpiece, in some varieties at least. Such a situation could not possibly be explained on the basis of poor graft unions alone. BRADFORD (**2**) apparently did not believe that all cases of dwarfing of grafted trees were the result of poor graft unions.

FRUITING RELATIONSHIPS

HATTON (**9**) made the interesting statement that of the Dr. Jules Guyot pear scions on clonal roots, "The two earliest cropping sets were on the two rootstocks that were among the most vigorous of the series." However, many of the trees of this variety were later found to have scion rooted. The Malling Twelve stock seems to be a remarkably barren, vigorous grower, in its first 15 years at least. Yet Whitney on Snow on Malling Twelve is rather dwarfish in growth, but still entirely unfruitful. The spurs appear to be low in N.

The fact that fruitfulness is so strikingly a variety characteristic in apples (Gravenstein, Spy, etc., have long been known as shy bearers and shy bloomers while Wealthy, Ben Davis, Baldwin, and Golden Delicious are known as heavy bearers as a rule) leads one to suspect that the scion (or even an inter variety) itself contains certain factors greatly affecting fruit bud formation. These factors may be photosynthetic rates in the scion, respiration (variety) rates, hormones, the degree of apical dominance (Loeb effect), and suppression of spur growth (reducing the number of spurs, as occurs in Spy and in the Malling Twelve stock itself). On these inherent factors are superimposed the nitrogen, mineral, and water nutritional variables the plant physiologist has dealt with so largely in the past. The remarkably high starch accumulation in the young roots of Wealthy trees in October is evidence of the unusual capacity of the Wealthy scion (table VI) for carbohydrate manufacture and storage (as starch).

It is obvious that a fair water supply to the top early in the season, with sharply decreased water later in the season (as supplied by dwarf roots), results in an early accumulation of both N and starch in the spurs and 2-year old branches, favoring abundant fruit bud formation (table VII). Properly ringed branches also show in August a high N, high starch, and low water content.

In shoots of trees of a given variety grown on Malling Nine or on Malling Twelve stock, the N content of the shoots is rather a constant for a given date in *early summer*. Later in the season shoots, 2-year old

branches, and spurs of trees dwarfed by the Malling Nine roots were much higher in N than similar parts of these varieties on Malling Twelve roots.

Spy shoot terminals from the inner shady parts (where the first fruit is often borne) of young Spy on seedling trees have the high N level characteristic of the fruitful Spy on Malling Nine shoot terminals in late July. The shoot terminals from the outer parts of young Spy trees have a low N level equal to that of Spy on Malling Twelve terminals at the same date.

Summary

1. Whitney, McIntosh, Spy, and Wealthy all show more or less dwarfing of top growth when grafted on the Malling Nine (Paradise) rootstock, compared with growth of the same varieties on the Malling Twelve root. Snow and York, two extremely high N varieties, show little or no dwarfing on Malling Nine roots, and grow only moderately well on Malling Twelve roots during their early years.

2. Roots of severely dwarfed trees on Malling Nine are not starved for starch, fat, or nitrogen compounds, but are well stocked with these reserves. The graft union of these dwarf trees did not prevent the downward movement of carbohydrate or soluble nitrogen compounds to the roots.

3. Upward movement of these reserve foods from the roots to the shoots in early summer seemed to be limited in dwarf trees, not because of poor xylem unions but probably as a result of early cyclic suberization of the small roots, giving a limited supply of water to the tops. Upward movement of organic reserves from the roots to the shoots occurred freely in the vigorously growing trees on Malling Twelve roots (and even in Snow on Malling Nine trees, where little dwarfing occurs, with the young roots showing an enormous development of large tracheae).

4. The use of Snow as an intermediate stem piece with Whitney top and Malling Nine root resulted in nearly emptying the root trunk of starch in early summer, and increased the growth of the Whitney top, as compared with Whitney on Malling Nine roots direct.

5. The use of Malling Nine as interpiece in Whitney on seedling root grafts resulted in extreme retention of organic reserves in the root below the Malling Nine interpiece. The use of a Malling Twelve interpiece resulted in free upward movement of the stored food in early summer.

6. Wealthy used as an interpiece in double-worked trees tended to make vigorous trees of most varieties, and resulted in shoot leaves higher in N than those of similar varieties worked on Jonathan intermediates.

7. Jonathan used as an interpiece tended to dwarf most varieties, and resulted in shoots higher in Fe and Mn and lower in N than the shoots of trees double-worked on Wealthy.

8. The Malling Twelve stock itself made much of its rapid shoot growth after the Malling Nine shoots had stopped terminal growth. Malling Nine root tip growth began before April 20, and the young roots were perhaps not entirely dormant during the winter months. Malling Twelve root tip growth began April 30, and vigorous root growth started about June 1. The root tips of Malling Nine in June were peculiar in shape, flattened, rather broad and blunt.

9. The Malling Nine stock is markedly lacking in apical dominance, the body of the tree being rather high in nitrogen while the shoots are low in this element.

10. Shoots of very fruitful dwarf trees on Malling Nine roots show by the new methods early (June 15 to July 1) accumulation of starch, while shoots of unfruitful vigorously growing trees on Malling Twelve roots do not show an early starch accumulation. These differences could not be shown by the taka-diastase methods, by malt diastase, nor by crude acid hydrolysis.

11. The starch method used is compared, as to results, with the calcium chloride iodide method of DENNY, and with the taka-diastase method.

12. Spurs and 2-year old branches of very fruitful dwarf trees on Malling Nine roots accumulate both nitrogen and starch as the season advances. Spurs and 2-year old branches of entirely unfruitful vigorous trees on Malling Twelve roots fall to a low nitrogen level as summer advances.

13. Spy shoot terminals from the inner shady parts (where the first fruit is often borne) of young Spy trees on seedling roots have the high nitrogen level characteristic of fruitful Spy on Malling Nine shoot terminals in late July. But the shoot terminals from the outer parts of young Spy trees have a low nitrogen level equal to that of the Spy on Malling Twelve terminals at the same date.

14. As originally suggested by GRUBB, long intermediate pieces of certain varieties probably would give valuable results in the production of uniform, hardy, high yielding apple trees. The extensive use of such trees as Virginia crab for top-working in the north central states seems entirely justified on the basis of physiological studies of double-worked apple trees.

15. Extremely heavy yielding varieties (Snow and York) on seedling roots are high in nitrogen not only at the shoot tips (as in Spy) but throughout the top and root as well.

The writer wishes to acknowledge the assistance given by Dr. R. H. ROBERTS, Prof. J. G. MOORE, and Dr. E. B. HART in the course of this research.

UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. BLACKMAN, V. H. Some physiological considerations in horticulture. Proc. 1st Imperial Hort. Conference. Part II. 37–41. London. 1930.
2. BRADFORD, F. C., and SITTON, B. G. Defective graft unions in apple and pear. Michigan Agr. Exp. Sta. Tech. Bull. 99. 1929.
3. BUNYARD, E. A. The history of Paradise stocks. Jour. Pomol. and Hort. Sci. **6**: 1–8. 1927.
4. DENNY, F. E. Improvements in the methods of determining starch in plant tissue. Contrib. Boyce Thompson Inst. **6**: 129–146. 1934.
5. ERLICH, F. Über die Pectolase, ein neuaufgefundenes Pektin ferment. Biochem. Zeit. **250**: 525–534. 1932.
6. GRUBB, N. H. East Malling Annual Report. General. **17**: 35. 1929.
7. HARVEY, E. M. A study of growth in summer shoots in apple with special consideration of the rôle of carbohydrates and nitrogen. Oregon Agr. Exp. Sta. Bull. 200. 1923.
8. HATTON, R. G. Paradise apple stocks. Jour. Roy. Hort. Soc. **42**: 361–399. 1917.
9. ————. General Review. East Malling Annual Report. **20**: 24. 1932.
10. HOOKER, H. D., JR. Certain responses of apple trees to nitrogen applications of different kinds at different seasons. Missouri Agr. Exp. Sta. Res. Bull. 50. 1922.
11. KNIGHT, R. C. Water relations of apples. East Malling Annual Report. **13**: 55–56. 1925.
12. NIEMANN, C. G., ROBERTS, R. H., and LINK, K. P. Isolation and determination of starch in plant tissue. Plant Physiol. **10**: 579–580. 1935.
13. NIGHTINGALE, G. T., and BLAKE, M. A. Effects of temperature on growth and composition of Stayman and Baldwin apple trees. New Jersey Agr. Exp. Sta. Bull. 566. 1934.
14. ROBERTS, R. H. Some stock and scion observations on apple trees. Wisconsin Agr. Exp. Sta. Bull. 94. 1929.
15. ————. Ring grafting and stock effect. Proc. Amer. Soc. Hort. Sci. **32**: 328–329. 1934.
16. ROGERS, W. S. Root studies. IV. Method of observing root growth in the field. East Malling Annual Report. **21**: 86–91. 1933.
17. SMALL, J. C. The quantitative determination of soluble starch in the presence of starch and its hydrolytic cleavage products. Jour. Amer. Chem. Soc. **41**: 107–112. 1919.

18. SULLIVAN, J. T. Estimation of starch in woody plants. Abstracts Amer. Soc. Plant Physiologists. Pittsburgh Meeting. 1934.
19. SWARBRICK, T. Factors governing fruit bud formation. Jour. Pomol. Hort. Sci. 7: 100–129. 1929.
20. TUKEY, H. B., and BRASE, K. D. Influence of the scion and of an intermediate stem-piece upon the character and development of roots of young apple trees. New York Agr. Exp. Sta. Geneva Tech. Bull. 218. 1933.

CHANGES IN NITROGEN COMPOUNDS IN THE WHEAT GRAIN AT DIFFERENT STAGES OF DEVELOPMENT[1]

GEORGE L. TELLER

(WITH TWO FIGURES)

Introduction

Marketable wheat is mostly considered as having reached a full stage of maturity. There is, however, much wheat on the market whose development has been checked at various stages short of full maturity. This is a somewhat variable condition, depending upon climate, season, soil, moisture, and time of harvesting as well as upon the type of grain. Much of this immature wheat has valuable, and under some conditions superior, milling qualities. The results of a systematic study of changes which take place during growth and development of the grain are therefore of practical value as well as of scientific interest.

ALSBERG, in considering the character of the proteins laid down during development, states (**1**, p. 236), "With the exception of WOODMAN and ENGLEDOW (**13**) almost no one has distinguished between the two proteins of which gluten is composed, glutenin and gliadin." ALSBERG draws this conclusion from the reading of BAILEY (**2**) and refers his readers to BAILEY for a review of the literature. BAILEY'S notes on this subject are not complete, although he gives the results of WOODMAN and ENGLEDOW in much detail. Their report, and the methods they used, show much to be desired. Among other things the recording of ammonia nitrogen should be explained. Even in the mature grain it is reported as high as 0.10 per cent. and higher. It is also clear that a large part of the gliadin was carried away in the 1 per cent. salt solution which they used before extraction with alcohol was begun. The amounts of gliadin shown, therefore, are much too low.

Methods

The writer made an extended study of wheat and its products during the years 1893 to 1898 inclusive. In 1893 researches of OSBORNE and VOORHEES (**7**) on the proteins of wheat were then new. Based upon their findings, convenient methods for making systematic series of determinations on the amounts of different nitrogen compounds in wheat and its products were desired. It was a pioneer undertaking and the nature of the substances to be separated does not tend toward exactness. After a careful preliminary study certain corrections for overlapping solubilities were

[1] Contribution from The Columbus Laboratories, Chicago, Illinois and the Arkansas Agricultural Experiment Station, Fayetteville, Arkansas.

found to be necessary. With these corrections the determinations on different wheats and products were found to be wholly consistent, and have been so found in numerous instances since that time.

These methods were first published in 1896 (**8**), and with some modifications again in 1898 (**9**). The original methods may be found in LEACH (**6**) and earlier editions where they were introduced by OSBORNE. They were used in studying the proteins of wheat bran (**11**) and are given in detail in the paper recording the results of that study.

Briefly, gliadin and the non-protein nitrogen compounds of wheat are soluble in neutral alcohol (sp. gr. 0.90). Little if any of the albumin and globulin are soluble in it. A 1 per cent. sodium chloride solution dissolves both of these, all the non-protein compounds, and some gliadin. Under uniform conditions the considerable amount of gliadin dissolved is very uniform (**8**). The protein compounds are completely precipitated from the salt solution by phosphotungstic acid, and the non-protein nitrogen in the filtrate can be readily determined. This must be deducted from the alcohol-soluble nitrogen to give the gliadin. The albumin and globulin may be precipitated from the salt solution by alcohol and their nitrogen determined in the precipitate as collected, or they may be found by difference when deductions for dissolved gliadin nitrogen and the non-protein nitrogen are made. The glutenin nitrogen is found as the difference between the total nitrogen and the sum of the nitrogen of the other compounds. Gliadin and glutenin are the two proteins which together form the greater part of gluten as it is ordinarily separated from flour by hand washing. All nitrogen determinations were made by the Kjeldahl method or the Gunning modification. The factor 5.7 was used for calculating protein from the nitrogen.

Experimentation

During the years 1897 and 1898, wheat at Fayetteville, Arkansas, was cut daily during the development of the grain and for several days after full ripeness. A portion of each cutting was put to dry on the straw in a well ventilated barn loft on a wire netting suspended from the roof to protect from rodents. From another portion the heads were cut and dried in like manner. In due time each lot was threshed by a threshing machine cylinder supported in a suitable wooden frame with box attachment to receive the threshed grain. This was cleaned in a farm fanning mill and stored in jars for analysis. During the first year a part of that dried on the straw was milled in a small roller mill to obtain the pure endosperm or fine flour for the analysis. This was carefully separated from the remainder by a series of hand sieves with bolting silks.

An extended analysis was made on the successive lots of wheat to determine total mineral matter, fat, different forms of carbohydrates, and

different nitrogen compounds. This paper for the most part deals only with the last. The methods used were those just described and the determinations included total nitrogen, non-protein nitrogen, the albumin and globulin (edestin and leucosin), the prolamine (gliadin), and the glutelin (glutenin). The number of kernels in 10 gm. of that ripened on the straw was also determined. The latter is a helpful guide to the condition as to development. The percentages of starch shown in table X contribute also to that end.

For convenient comparison of data the results of analyses of the several daily cuttings are grouped as the average of three days each. However, the wheat from each day was analyzed separately for the nitrogen and its compounds, both that dried on the straw and that in the head cut from the straw. For other determinations the three-day samples were mixed before analyses and the analyses were made only on those dried on the straw.

Detailed results of these several analyses of the grain of the harvest of 1897 have already appeared (**9**). Results of the protein separations for 1898 have not heretofore been published, although the more general analyses were published in a paper discussing carbohydrates (**10**). Results of both years are given here as either without the other would lose some-

TABLE I

APPROXIMATE STAGE OF DEVELOPMENT OF WHEAT WHEN CUT AS RECORDED FOR PERIODS OF THREE DAYS EACH. SERIES OF 1897

PERIOD	STAGE OF DEVELOPMENT
I	A little past blossom; grain set
II	Berries one-half to full length of ripe grain
III	Crushed berries exude a thin milky liquid; lower leaves beginning to die
IV	Grain well in milk
V	Heads and kernels well developed; interior of grain a thin dough
VI	Grain in dough
VII	Grain in stiff dough; straw becoming yellow at butt; grain shells a little with rough handling
VIII	Straw in field much yellowed but still decidedly green
IX	Grain oozes a thin liquid when crushed between the thumb nails; contents still slightly viscid; straw still a little green
X	Wheat fit to cut at beginning of this period; straw has lost all its green color and is dark purple immediately below the heads; berry nearly dry and may be crushed between the thumb nails but without contents adhering to them
XI	More than ripe; straw bright and stands up well
XII	More than ripe; straw bright and stands up well
XIII	More than ripe; straw bright and stands up well
XIV	More than ripe; straw bright and stands up well

thing of its value. The comparisons as shown in the charts (figs. 1, 2) are especially instructive.

TABLE II

NUMBER OF KERNELS IN 10 GM. OF WHEAT RIPENED ON STRAW

PERIOD	SERIES OF 1897	SERIES OF 1898
I	3125	9632
II	1964	4628
III	1080	1708
IV	746	834
V	494	519
VI	412	426
VII	333	362
VIII	296	321
IX	269	298
X	254	293
XI	253	293
XII	252	285
XIII	247	286
XIV	248	.....

TABLE III

AVERAGE TOTAL NITROGEN IN PERCENTAGE DRY MATTER IN WHEAT GRAIN DRIED ON THE STRAW AND IN HEADS CUT FROM THE STRAW. SERIES OF 1897

PERIOD	HEAD SERIES	STRAW SERIES	DIFFERENCE
	%	%	%
I	3.85	3.68	0.17
II	3.63	3.25	0.38
III	3.20	2.85	0.35
IV	2.90	2.63	0.27
V	2.73	2.55	0.18
VI	2.45	2.36	0.09
VII	2.64	2.45	0.19
VIII	2.73	2.69	0.04
IX	2.75	2.78	–0.03
X	2.95	2.95	0.00
XI*	3.01	2.91	0.10
XII	3.11	3.02	0.09

* Average two days only.

TABLE IV

AVERAGE TOTAL NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN				
	TOTAL	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%	%
I	3.68	1.04	1.63	0.44	0.57
II	3.25	1.13	1.40	0.44	0.28
III	2.85	1.14	1.08	0.43	0.20
IV	2.63	1.10	0.95	0.40	0.18
V	2.55	1.10	0.91	0.38	0.16
VI	2.36	1.08	0.84	0.33	0.11
VII	2.45	1.19	0.88	0.28	0.10
VIII	2.69	1.35	0.96	0.28	0.10
IX	2.78	1.49	0.91	0.29	0.09
X	2.95	1.58	0.93	0.34	0.10
XI	2.91	1.54	0.94	0.34	0.09
XII	3.02	1.62	0.94	0.35	0.11
XIII	3.03	1.59	0.98	0.34	0.12
XIV	3.17	1.68	1.01	0.36	0.12

TABLE V

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN			
	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%
I	28.4	44.2	11.9	15.5
II	34.7	43.1	13.7	8.5
III	39.9	37.8	15.1	7.2
IV	41.9	36.2	15.2	6.7
V	43.3	35.7	14.7	6.3
VI	45.6	35.6	13.9	4.9
VII	48.5	35.8	11.4	4.3
VIII	50.0	35.9	10.3	3.8
IX	53.5	32.7	10.4	3.4
X	53.3	31.5	11.8	3.4
XI*	52.9	32.4	11.5	3.2
XII	53.5	31.1	11.5	3.9
XIII	52.4	32.3	11.1	4.2
XIV	53.0	31.9	11.3	3.8

* Average two days only.

TABLE VI

AVERAGE TOTAL NITROGEN AND NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1898

PERIOD	NITROGEN				
	TOTAL	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%	%
I	4.45	0.43	1.41	0.28	2.33
II	3.84	0.93	1.70	0.45	0.76
III	3.17	1.18	1.23	0.50	0.26
IV	2.67	1.09	0.97	0.44	0.17
V	2.56	1.11	0.90	0.41	0.14
VI	2.60	1.23	0.86	0.39	0.12
VII	2.66	1.29	0.86	0.39	0.12
VIII	2.65	1.31	0.87	0.37	0.10
IX	2.61	1.31	0.82	0.38	0.10
X	2.62	1.31	0.83	0.38	0.10
XI	2.61	1.33	0.81	0.37	0.10
XII	2.58	1.30	0.82	0.37	0.09
XIII	2.59	1.30	0.84	0.36	0.09

TABLE VII

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1898.

PERIOD	NITROGEN			
	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%
I	9.6	31.7	6.3	52.4
II	24.2	44.3	11.7	19.8
III	37.2	38.8	15.8	8.2
IV	40.8	36.3	16.5	6.4
V	43.3	35.2	16.0	5.5
VI	47.3	33.1	15.0	4.6
VII	48.5	32.3	14.7	4.5
VIII	49.4	32.9	13.9	3.8
IX	50.2	31.4	14.6	3.8
X	50.0	31.7	14.5	3.8
XI	51.0	31.0	14.2	3.8
XII	50.4	31.8	14.3	3.5
XIII	50.2	32.4	13.9	3.5

TABLE VIII

AVERAGE TOTAL NITROGEN AND NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL DRY MATTER OF ENDOSPERM OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN				
	TOTAL	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%	%
VI	2.03	1.19	0.62	0.19	0.02
VII	2.08	1.20	0.68	0.18	0.02
VIII	2.18	1.31	0.67	0.18	0.02
IX	2.21	1.33	0.68	0.18	0.02
X	2.31	1.41	0.70	0.18	0.02

TABLE IX

AVERAGE NITROGEN OF DIFFERENT COMPOUNDS IN PERCENTAGE TOTAL NITROGEN IN ENDOSPERM OF WHEAT GRAIN CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH. GRAIN DRIED ON THE STRAW. SERIES OF 1897

PERIOD	NITROGEN			
	GLIADIN	GLUTENIN	EDESTIN AND LEUCOSIN	NON-PROTEIN
	%	%	%	%
VI	58.6	30.8	9.6	1.0
VII	57.8	32.7	8.2	1.0
VIII	60.1	30.8	8.2	0.9
IX	60.2	30.8	8.1	0.9
X	61.0	30.3	7.8	0.9

Discussion

As shown in table II, there were more than three times as many kernels in the same weight of grain in the first period of the second year as in the same period of the first year. This striking difference is apparently due to at least four days' earlier cutting in the stage of growth. This is also apparent in the results of the analyses, especially as to the much higher nitrogen content and the much greater proportion of non-protein nitrogen in the first period of the second year's cutting.

Comparing the total nitrogen in the grain ripened on the straw and that in the heads cut from the straw (table III), we find a material decrease in the nitrogen in the former. This is apparently to be attributed to the transfer of carbohydrate substances from the straw into the grain. In

TABLE X

TOTAL NITROGEN AND STARCH* IN PERCENTAGE TOTAL DRY MATTER OF WHEAT CUT DAILY AND ARRANGED IN PERIODS OF THREE DAYS EACH DURING TWO HARVEST YEARS. GRAIN DRIED ON THE STRAW

SERIES OF 1897			SERIES OF 1898		
PERIOD	TOTAL NITROGEN	STARCH	PERIOD	TOTAL NITROGEN	STARCH
	%	%		%	%
I	3.68	41.5	I	4.45	16.7
II	3.25	47.7	II	3.84	29.7
III	2.85	54.7	III	3.17	47.2
IV	2.63	59.4	IV	3.67	56.4
V	2.55	63.8	V	2.56	60.1
VI	2.36	65.3	VI	2.60	61.8
VII	2.45	65.7	VII	2.66	62.1
VIII	2.69	65.5	VIII	2.65	62.1
IX	2.78	65.4	IX	2.61	62.9
X	2.95	64.3	X	2.62	63.4
XI	2.91	65.2	XI	2.61	64.5
XII	3.02	64.4	XII	2.58	63.5
XIII	3.03	63.8	XIII	2.59	63.1
XIV	3.17	63.2			

* The starch as here shown corresponds closely to the amount found by diastase followed by acid hydrolysis but includes also a small amount of undetermined material, especially in the early periods.

the second year's cutting there was not such a marked and constant difference and the results are not here shown. The relations of the different forms of nitrogen compounds in percentage of total nitrogen in the straw series and in the head series were much the same during each year.

There was a steady decrease in percentage of nitrogen in the grain during the early period of both years (tables IV and VI). Following this there was during the first year a steady and marked increase up to and including the final cutting. During the second year this increase was hardly more than begun when a decrease set in and continued to the end. Since these cuttings were made in nearly the same location and on the same variety of wheat (Fulcaster), it is clearly a seasonal difference. This decrease in nitrogen in the grain was found by KEDZIE (**4, 5**) in two years' cuttings in Michigan (1881 and 1893). An increase in nitrogen similar to that of the first year was found by BRENCHLEY and HALL (**3**) at Rothamsted, England, and by THATCHER (**12**) on American spring wheat. This variability is significant in connection with preliminary surveys of the nitrogen content of the coming wheat crop as now sometimes made from early sampling in the field.

The material increase of nitrogen in the endosperm or flour portion of the grain over the periods during which this material was separated is of even greater interest (table VIII). The percentage of total nitrogen in the dry matter is more than one-eighth greater in period V than in period VI.

In separating the endosperm an effort was made to secure only the purest of it. In doing this a large proportion of good flour material was rejected. That portion of the flour rejected lies closer to the bran and contains a distinctly larger proportion of nitrogen than that near the center of the kernel. Because of this the difference between the percentage of nitrogen in the endosperm as examined and in the whole wheat is greater than it would have been if all the endosperm had been included. When we compare the amount of gliadin in the endosperm in percentage of the total nitrogen, however, we find it greater than in the wheat. This is fully in accord with my previous findings made in 1896 and in 1932, which show a markedly higher proportion of gliadin in the endosperm than in the bran. The steady increase of gliadin in the wheat during the early stages of both years (tables V and VII; figs. 1, 2) is due in large measure to the increased filling of the grain with endosperm. It parallels somewhat closely the increase in the proportion of starch as shown in table X. That

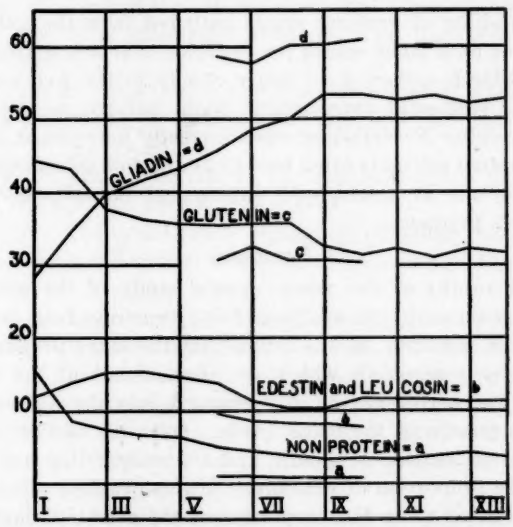


FIG. 1. Changes in proportions of nitrogen compounds in grain of wheat from early formation to past maturity; series of 1897. Short lines with letter indicate corresponding compounds in endosperm; figures show percentage; Roman numerals indicate period.

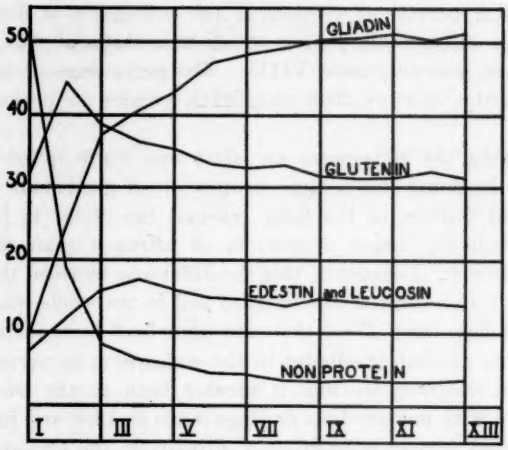


FIG. 2. Changes in proportions of nitrogen compounds in grain of wheat from early formation to past maturity; series of 1898. Figures show percentage; Roman numerals indicate period.

it is not wholly due to this cause is apparent from tables VIII, IX, and others.

The composition of growing plants gathered from the soil at different times is subject to so many causes for variation that it is extremely difficult if not impossible to adhere even fairly closely to the well established requirements of successful experimental work, namely, to allow variations along one line only. Nevertheless, when correctly interpreted, the combined results of repeated attempts often lead to findings of substantial value. At all events they are in accord with nature and the industries that make use of nature's products.

Summary

1. Among results of two years' careful study of the development of wheat grain, as shown by the analyses of daily cuttings from its first formation to far past maturity, it was found that the large proportion of non-protein nitrogen compounds which are predominant at the very earliest formation of the kernel are quickly changed into the different forms of protein characteristic of the wheat grain. Over the earlier periods there was a continuous increase of gliadin and a corresponding but less marked decrease in the proportion of glutenin. In these earlier periods there was also a slight falling off in the proportion of the combined non-gluten proteins, the albumin and globulin. These changes are in part due to the filling of the grain with endosperm. This contains a much larger proportion of gliadin and a smaller proportion of glutenin and the non-gluten nitrogen compounds than the bran.

2. For both years there was a decrease of total nitrogen in the grain over the early periods, which is also in accord with the results of increased endosperm. Owing to seasonal differences there was for one year a steady increase in the proportion of total nitrogen in the grain throughout the later periods. For the other year there was a decrease in this proportion of nitrogen. Both of these conditions as to the relative amounts of total nitrogen in the grain are in conformity with findings of certain other investigators who have pursued that feature of the study.

THE COLUMBUS LABORATORIES
CHICAGO, ILLINOIS

LITERATURE CITED

1. ALSBERG, C. L. The physiology of the wheat plant. Wheat studies of the Food Research Inst. Stanford Univ. Calif. **10**: 236. 1934.
2. BAILEY, C. H. Growth and development of wheat. The chemistry of wheat flour. pp. 42–48. 1925.
3. BRENCHLEY, WINIFRED E., and HALL, A. D. The development of the grain of wheat. Jour. Agr. Sci. **3**: 195–217. 1909.
4. KEDZIE, R. C. The ripening of wheat. Rept. Michigan Bd. Agr. 233–238. 1881–1882.
5. ————. Composition of the grain and straw of wheat at different periods of ripening. Michigan Agr. Exp. Sta. Bull. 101. 2–12. 1893.
6. LEACH, ALBERT E. Separation and determinations of wheat proteins, TELLER'S method. Food Inspection and Analysis. 4th ed. p. 307. 1920.
7. OSBORNE, T. B., and VOORHEES, C. G. Proteids of the wheat kernel. Amer. Chem. Jour. **15**: 392–471. 1893.
8. TELLER, G. L. The quantitative separation of wheat proteids. Concerning wheat and its mill products. Arkansas Agr. Exp. Sta. Bull. 42. Part 2. 81–104. 1896.
9. ————. A report of progress of investigations in the chemistry of wheat. Arkansas Agr. Exp. Sta. Bull. 53. 53–81. 1898.
10. ————. The carbohydrates of wheat and wheat products and changes in same during development of the grain. Orig. Communication 8th Internat. Cong. of Appld. Chem. **13**: 273–278. 1912.
11. ————, and TELLER, W. K. A study of proteins of wheat bran. Cereal Chem. **9**: 560–572. 1932.
12. THATCHER, R. W. The progressive development of the wheat kernel. II. Jour. Amer. Soc. Agron. **7**: 273–282. 1915.
13. WOODMAN, H. E., and ENGLEDOW, F. L. A chemical study of the development of the wheat grain. Jour. Agr. Sci. **14**: 563–586. 1924.

DISTRIBUTION OF ROOTS IN POROUS AND NONPOROUS PLANT CONTAINERS[1]

LINUS H. JONES AND HENRI D. HASKINS

(WITH THREE FIGURES)

Introduction

Many years of experience in growing plants in clay pots have taught the horticulturist to judge the needs of his plants by the condition of the root system. The so-called pot-bound condition, in which the root system appears as a mass of interlacing roots between the pot and the soil mass, is a criterion that is applicable only to this type of container, *i.e.*, the porous clay pot.

JONES (1) has shown that plants may be grown in nonporous containers and that such plants are equal to those grown in the conventional clay flower pot. The study of root systems in both porous and nonporous plant containers shows a striking difference. In the porous pot, the root system is almost wholly outside the soil mass and the core of soil within may be easily removed. In the nonporous pot, there are relatively few roots on the outside of the soil mass and it is exceedingly difficult to remove the soil from the roots, which ramify throughout the soil. This condition was observed by WHITNEY and CAMERON (7) in 1904 when experimenting with soils in wire baskets covered with paraffin. Such plant containers are nonporous. These investigators state: "In no case has there been found any evidence of any effort on the part of roots to develop toward the sides of the pot. On the contrary, they grow freely throughout the soil." The wire basket covered with paraffin was the outcome of failures to keep the root system within the soil, for in glass tumblers this particular soil contracted, leaving an air space saturated with water vapor between the soil and the wall of the tumbler. These investigators further noted that they obtained an even distribution of soil moisture within these paraffined wire baskets.

Top-root ratios in porous and nonporous plant containers

A comparison of the root systems of plants grown in porous and in nonporous pots invariably showed a larger root system in the porous pot; moreover, it was observed that the soil in nonporous pots always contained a greater amount of moisture than did the soil in porous pots.

In order to obtain more definite information on top-root ratios of plants grown in porous and nonporous containers, the following experiment was carried out.

[1] Contribution no. 186 of the Massachusetts Agricultural Experiment Station.

Sixty 3-inch clay pots and sixty glass tumblers, each holding the same volume of soil as a 3-inch clay pot, were planted with tomato seedlings. The containers were watered each day and checked every alternate day by weighing in order to maintain a fairly even moisture content of the soil. The soil in the clay pots, however, frequently became dry owing to excessive evaporation from the walls of the pots. It was necessary to apply twice as much water to the clay pots as was applied to the tumblers. At the end of 33 days, the plants in the clay pots were showing signs of becoming pot-bound, *i.e.*, the roots were heavily massed against the wall of the pot and even projected above the surface of the soil at the region of contact of soil and pot. At this time the soil was carefully washed from the roots, and the top of each plant was cut off just above the first root. The tops and roots were dried in an oven at 101° C. and weighed. Table I gives the average weights obtained.

TABLE I

AVERAGE DRY WEIGHTS OF 60 TOMATO PLANTS GROWN IN CLAY POTS AND OF 60 TOMATO PLANTS GROWN IN GLASS POTS FOR 33 DAYS

TYPE OF POT	AVERAGE WEIGHT OF ROOTS	AVERAGE WEIGHT OF TOPS	AVERAGE TOTAL WEIGHT OF PLANT	AVERAGE T/R RATIO
	gm.	*gm.*	*gm.*	
Clay	0.3853	1.2750	1.6603	3.31
Glass	0.3429	1.3773	1.7202	4.02

Although an attempt was made to maintain a fairly uniform moisture content in the soil, observations indicated that only by frequent weighings over the daily period could the moisture in the soil of the clay pots approximate the uniform moisture content of the glass tumblers. The result of this experiment, as shown by table I, demonstrates that a somewhat smaller root system in the glass container produced fully as large a plant as was obtained in the clay pot. However, the difference was slight in the total weights of plants (roots and tops) grown in both types of containers, although as a matter of fact the average weight of the whole plant grown in glass was somewhat greater than that obtained in clay. As noted in previous experiments, the root systems were distributed according to the type of container in which they were growing, the roots in the clay pot being for the most part massed on the outside of the soil and those in the glass tumbler being well distributed throughout the soil mass. Figure 1 illustrates these two types of root distribution and figure 2 shows the two root systems with soil removed.

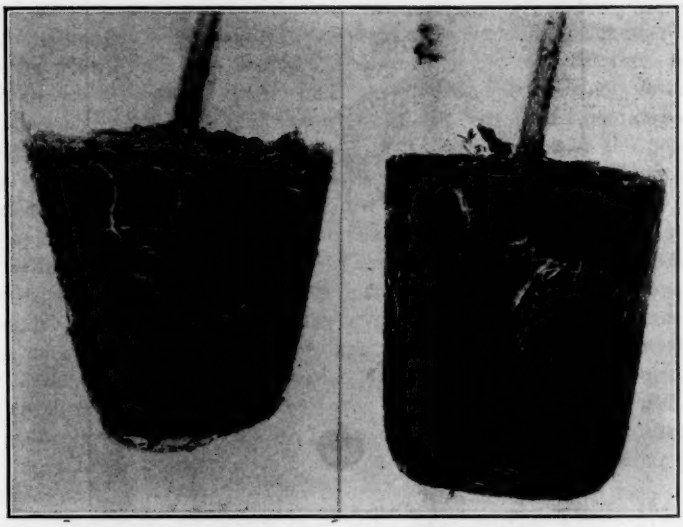


FIG. 1. Distribution of roots in porous pot (left) and nonporous pot (right). Tomato plants 40 days old.

Relation of nutrients to root distribution

It is generally believed that roots grow between the wall of a clay pot and the soil mass in order to obtain the air that is supposed to pass through the wall of this type of plant container. In fact, it is the presence of the root system at this point of contact between pot and soil that is offered as proof that air does pass through such walls. The demonstration by JONES (**2**) that a rubber vacuum disc will remain fixed on the moist wall of a clay pot for over 30 days disproves the popular belief that a clay flower pot is porous to air. It is necessary, therefore, to find some other explanation for the massing of roots near the wall of the clay pot.

There is considerable evidence in the literature to show that under normal moisture conditions root formation takes place where there is an available source of nutrients, and, up to a certain point, the amount of root formation increases directly as the nutrient supply increases. LIVINGSTON (**4**) found an increased branching of roots as the amount of manure was increased in a poor soil. The tremendous development and branching of roots within drain tiles, even in regions where there is plenty of soil moisture, is a response to a nutrient factor. It has been noted that an unusually great number of roots will develop in manure layers where manure is used alternately with soil, or where a layer of clay has absorbed some of the nutrients that have leached through sand.

FIG. 2. Root systems of figure 1: left, porous pot; right, nonporous pot.

The porous pot not only absorbs moisture, but it also provides a means of moving soil moisture into the air through the wall of the pot. Such pots are capable of losing twice as much water through the wall of the pot as from the surface of the soil. A theory that the distribution of roots in plant containers is tied up directly with plant nutrients and is indirectly related to soil moisture movements is partly supported by the following observations and chemical analyses.

The work of KNOTT and JEFFRIES (3) indicates the large amount of nitrogen that is absorbed by the clay pot from the soil within. In the opinion of the writers, roots develop at the point where the relatively large amount of available nitrogen is concentrated by the movement of the water drawn through the wall of the pot by the force of evaporation. The successful horticultural practice of using small pots for small plants may be explained on the basis that in the small pot the roots can more quickly reach this source of nutrients. A small plant in a large pot grows slowly

compared with a small plant in a small pot. The practice of placing poorly rooted cuttings nearer the wall of the pot than the center in order to encourage better growth may be interpreted to mean that the roots are there much nearer the region where nitrogen and other nutrients will be concentrated. Tests have confirmed these practices with the clay pots; but when nonporous pots of paper or glass were employed, the size of the pot or the position of the seedling or cutting had no effect on subsequent growth, and this growth was equal to any growth obtained in the clay pots.

It appears that the soluble salts in the soil, which include the nutrients, move in the same direction as the moisture. The marked lateral movement of moisture that occurs in a clay pot is entirely absent in a nonporous pot. Although vertical movement of moisture occurs in both the clay and nonporous pots, any upward movement of salts is counteracted by top watering which leaches these salts downward. The more even distribution of soil moisture in the nonporous pots compared with moisture distribution in clay pots probably has associated with it a more even distribution of nutrients, which may account for the ramifying root systems in the nonporous plant containers.

A demonstration that there is an unequal distribution of nutrients in clay flower pots is shown in the following results, which are taken from some preliminary experimental work that bears upon the subject of lateral movement of nutrients in the soil of a clay flower pot. To each of six 6-inch flower pots containing soil were added 7.5 gm. of a 5–8–7 fertilizer, practically all of which was in a soluble form. The soil was kept fallow and the pots watered each day. The pots were kept on a bed of moist cinders. At intervals of 4, 8, and 12 weeks, two pots were sampled in four cores according to the following diagram (fig. 3). Portions of each sample were washed with like amounts of distilled water, the solutions evaporated, and a determination made of the total water-soluble solids. It was found that the greatest concentration of water-soluble solids was always obtained in the center core (*A*), and that in general there was a definite reduction of total water-soluble solids progressing through cores *B*, *C*, and *D*. Each of these cores

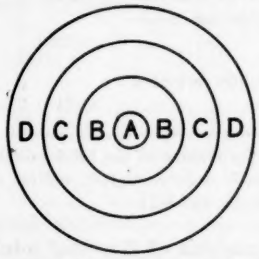


FIG. 3. Diagram showing relative positions of cores *A*, *B*, *C*, and *D*.

contained less total water-soluble solids as the sample taken became closer to the wall of the pot. In table II are given the results obtained from the 4-, 8-, and 12-week samples.

TABLE II

TOTAL WATER-SOLUBLE SOLIDS IN THE SEVERAL CORES DRAWN FROM 6-INCH POROUS CLAY POTS. RESULTS CALCULATED TO A DRY MATTER BASIS

	TOTAL WATER-SOLUBLE SOLIDS			
	CORE A	CORE B	CORE C	CORE D
	%	%	%	%
4-week samples	0.8306	0.6144	0.5229	0.3899
8-week samples	0.9368	0.5878	0.5646	0.3941
12-week samples	0.7710	0.4650	0.5816	0.4364

The detailed analysis of a composite of the total water-soluble solids from the several cores, drawn at the three intervals, is given in table III.

TABLE III

DETAILED ANALYSIS OF COMPOSITE SAMPLES OF CORES DRAWN AFTER 4-, 8-, AND 12-WEEK INTERVALS. RESULTS COMPUTED TO A DRY BASIS OF THE WATER-SOLUBLE SOLIDS

	CORE A	CORE B	CORE C	CORE D
	%	%	%	%
Total nitrogen	0.0113	0.0076	0.0101	0.0046
Nitrogen as nitrates	0.0082	0.0045	0.0067	0.0018
Nitrogen other than nitrates	0.0031	0.0031	0.0034	0.0028
Potash	0.1022	0.0704	0.0832	0.0554
Phosphoric acid	0.0054	0.0042	0.0028	0.0028
Calcium oxide	0.1627	0.0947	0.0765	0.0392
Magnesium oxide	0.0390	0.0257	0.0242	0.0189
Chlorine	0.0282	0.0203	0.0255	0.0216
Soluble sulphates (SO_3)	0.2743	0.1638	0.1410	0.0689
Total of soluble constituents as analyzed	0.6231	0.3867	0.3633	0.2114
Residue of total soluble solids not recovered by analysis*	0.2230	0.1690	0.1931	0.1954
Percentage of total soluble solids recovered by analysis	73.64	69.59	65.29	51.97

* It seems probable that the residue of the total soluble solids not recovered by these analyses would be largely soluble organic matter, sodium compounds, and small amounts of such substances as Mn, Fe, Al, and SiO_2.

This table shows in the analysis of the total soluble solids, the sum of the seven determinations on composite sample *A* was 66 per cent. more than on

sample *D;* about 42 per cent. more than on sample *C;* and 38 per cent. more than on sample *B*. This shows a diminishing quantity of soluble soil constituents from the center core (*A*) to the outer soil region (*D*), and agrees with the data in table II which show the percentages of total water-soluble solids from each sample. The data in these two tables indicate that there is a pull of soil salines toward the pot wall, *i.e.*, lateral diffusion, and that the pot has absorbed a considerable quantity of the soluble nutrients. The fact that core *D* contains a smaller quantity and cores *C* and *B* a somewhat larger quantity of the water-soluble constituents might indicate that these compounds had been absorbed by the pot walls. Naturally, as the concentration of the soil solution was less in the outer layers of the soil, in the presence of a lateral movement of soil moisture induced by the heavy pull caused by evaporation through the pores of the pot the supply of soluble nutrients must naturally come from cores *B* and *A*, which function as reservoirs for both water and the soluble constituents which the water carries.

That the pot does absorb soluble plant food constituents is illustrated by the chemical analysis (given in table IV) of a gray, crystalline deposit scraped from the outer surface of clay pots which had seen several years of service in a greenhouse.

The literature that might bear directly on this subject suggests that a plant container with an evaporating wall may cause a lateral diffusion of soil salines. McHargue (**5**), working with manganese, discusses this point.

TABLE IV

CHEMICAL ANALYSIS OF CRYSTALLINE DEPOSIT SCRAPED FROM SURFACE OF CLAY POTS

	%
Moisture in vacuum oven at 55° C. for 10 hours	2.30
Organic and volatile matter exclusive of free moisture	24.90
Total nitrogen	0.55
Ammoniacal nitrogen	None
Nitrate nitrogen	0.32
Water-soluble organic nitrogen	0.05
Water-insoluble organic nitrogen	0.18
Phosphoric acid	0.79
Iron and aluminum oxides	0.76
Calcium oxide	26.28
Magnesium oxide	1.08
Sulphates (SO_3)	35.09
Potassium oxide	0.39
Sodium oxide	0.89
Soluble silica	0.40
Silica insoluble in dilute hydrochloric acid (most likely a part of the clay pots)	7.20

He found that crocks that were not well glazed showed crystalline deposits of nutrients after the crocks had been in use a short time. The poor glazing permitted the migration of moisture which carried the mineral nutrients in solution through the walls, so that subsequent evaporation of the moisture caused deposition of the mineral nutrients on the outside. In a later publication McHargue (6) states that "the wall of earthenware jars which are in general use for pot experiments may be sufficiently porous to absorb mineral nutrients from soil or sand cultures, and these may become available and affect the growth of plants in subsequent experiments." Knott and Jeffries (3) found that new flower pots absorbed considerable nitrogen, the pot competing more successfully for this element than the plant. They also suggest that other soil salts may be similarly absorbed.

These ideas are well confirmed by the behavior of plants, particularly of their root systems, in the cement flower pot. The pore spaces in the wall of the cement pot are so large that only when the soil within is excessively moist is there a movement of capillary moisture through the wall of the pot. During the greater part of the time, therefore, the wall of the cement pot is dry and porous to air when supporting plant growth, and for this reason the root system ramifies through the soil and is not found between the soil and the pot. The cement pot, typifying these characteristics, supplies a basis for believing that root distribution is not dependent on nor greatly influenced by aeration through the wall of the pot. The ramifying root system found in both cement and nonporous containers is associated with a lack of lateral movement of soil moisture, which in turn influences a lack of lateral movement of soluble plant food.

In the porous clay pots, however, movement of capillary water through the wall of the pots and the presence of deposited salts left on the pots by the evaporated water result in massing of root systems between the soil and the pot. As reported by Knott and Jeffries (3), a new pot produces a stunted, yellow, inferior plant, as the wall of the pot absorbs a considerable portion of the soil nutrients. The moisture relations in a new and an old clay pot are the same, but the nutrient relations are different. It is reasoned from the evidence presented, therefore, that the presence of nutrients is the most important factor in root development, and that distribution of nutrients is related to soil moisture movements.

Summary

1. Root systems in porous pots are found, for the most part, outside the soil mass and next to the wall of the pot. In nonporous containers the root systems ramify throughout the soil with but a very small proportion next to the wall of the plant container.

2. The size of a root system is influenced by the moisture content of the soil. If the moisture content of the soil in a porous pot is maintained approximately equal to that in a nonporous container, the root systems will be nearly equal.

3. A determination of water-soluble solids made at intervals of 4, 8, and 12 weeks showed an unequal distribution of this material in the porous pots. In general, the amount of water-soluble solids decreased directly as the concentric cores were measured from the center to the periphery.

4. A detailed analysis of the composite samples of the four cores showed that all the elements for which determinations were made followed the same general rule of decreasing in concentration from the center outward.

5. The cement pot is porous to air, but does not maintain a lateral movement of moisture. The root system in a cement pot ramifies throughout the soil as does the root system in a nonporous pot.

6. The distribution of root systems in plant containers is associated with the distribution of nutrients, which in turn is affected by soil moisture movements.

MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION
AMHERST, MASSACHUSETTS

LITERATURE CITED

1. JONES, L. H. Flower pot composition and its effect on plant growth. Massachusetts Agr. Exp. Sta. Bull. 277. pp. 147–161. 1931.
2. ————. Aeration of soil in plant containers. Florists Exch. & Hort. Trade World **79**: no. 11, p. 39. March 12, 1932.
3. KNOTT, J. E., and JEFFRIES, C. D. Containers for plant growing. Pennsylvania Agr. Exp. Sta. Bull. 244. 1929.
4. LIVINGSTON, B. E. Note on the relation between growth of roots and of tops in wheat. Bot. Gaz. **41**: 139–143. 1906.
5. MCHARGUE, J. S. Common earthenware jars as a source of error in pot experiments. Jour. Agr. Res. **26**: 231–232. 1923.
6. ————. Manganese and plant growth. Ind. & Eng. Chem. **18**: 172–175. 1926.
7. WHITNEY, M., and CAMERON, F. K. Investigations in soil fertility. U. S. Dept. Agr. Bur. Soils Bull. 23. 1904.

THERMAL CONDUCTIVITY OF STORED OATS WITH DIFFERENT MOISTURE CONTENT[1]

A. L. BAKKE AND H. STILES[2]

(WITH ONE FIGURE)

Much has been written about the heating that takes place in damp or moist grain, but apparently no measurements have been made of thermal conductivity. In a previous publication by BAKKE and NOECKER (**1**), it was shown that in oats in storage there is considerable variation in oxygen consumption even with oats of low moisture content. There is a general tendency for respiration to increase with the moisture content, although there is considerable variation. In certain localized areas where the grain is not sufficiently dry for storage, spoilage takes place owing to rapid oxidation of the grain. In these areas or "heat pockets" the heat generated has been largely retained owing to the fact that the grain is a poor conductor of heat. The work reported in this paper is an attempt to measure the thermal conductivity of oats stored at different moisture contents.

Stiles's method of measuring thermal conductivity

The thermal conductivity of oats was measured by the apparatus developed by STILES (**2, 3**) in obtaining the thermal conductivity of heat-insulating materials. Briefly the method is to place the material to be tested between the ice vessel and the hot water tank, being sure that the upper temperature is constant at 32° F. Heat passage through the material to be tested is then determined by timing the water-level drop in the measuring tube which depends on the rate of melting of the ice in the cylinders.

The tank is a copper cylinder 14½ inches high and 16 inches in diameter, holding approximately 100 pounds of water. The water is heated by a Bunsen burner. On account of the large heat capacity of the water, it has been found possible after a little practice to keep the temperature of the water constant to within one-half degree for hours. The water is kept in circulation by a stirrer which consists of two sets of vanes mounted on a short shaft placed in a brass cylinder 3 inches in diameter. The pulley at the top of the shaft is belted to a one-eighth horsepower motor and the water circulated through holes near the top and near the bottom of the cylinder. The cylindrical sides of the boiler and ice container are of gal-

[1] Journal paper no. J 264 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project no. 87.

[2] Gratitude is expressed for the assistance given by Dr. I. E. MELHUS in the preparation of this manuscript.

vanized iron lagged with asbestos. The bottom of each container and the top of the boiler are brass plates $\frac{3}{32}$ of an inch in thickness; a hole near the top provides for the insertion of the thermometer.

By means of a cylinder of bakelite co-axially placed, the ice container is divided into two compartments, the outer one, which is about 3 inches wide, serving as a guard ring. Bakelite is used on account of its low thermal conductivity, lessening the possibility of transfer of heat into the cylinder. The cylinder is mounted on a short thin brass ring which is soldered to the bottom of the container. The top of the cylinder is machined out for a short distance down on the inside so as to contain a brass sleeve, which is threaded to mesh with the threads of the edge of the brass disk serving as a lid for the cylinder. The lid is slightly concave on the lower surface and has a hole in its center into which may be fitted a one-holed rubber stopper. Into this stopper is passed a glass tube the upper part of which has two bulbs. Two fine index marks are etched on the tube, one above and the other below the lower bulb. Further details concerning the apparatus may be secured by referring to the original articles.

In series 2 and 3 the oats were not weighed. Whatever oats were needed to fill the space between the two layers were used. In series 1 and 2 the thickness of the layer was 0.94 cm. In part of series 3 the layer was 1.48 cm. in thickness.

The general procedure has been adequately given by the junior writer and need not be repeated here. In order to make the calculations clear, however, it may be well to define some of the terms employed.

In making measurements in c.g.s. units, the specific gravity of ice is taken as 0.9164. For every 0.9164 gm. ice melted in the cylinder there is a volume shrinkage of approximately 0.0836 cc., and the water in the tube would decrease by that amount.

The volume of ice water contained by the lower bulb between the two marks of one of the tubes used was determined and for one of the tubes was found to be 3.87 cc. Therefore $(3.87 \div 0.0836) \times 0.9164$ equals the number of grams of ice melted in the cylinder during the test. With the heat of fusion of ice taken as 79.8 calories per gram, the total quantity of heat, Q, passing into the cylinder during the test was $(3.87 \div 0.0836) \times 0.9164 \times 79.8 = 3385$ calories. This figure is taken as a constant for all tests made with a particular tube. The thermal conductivity, k, of the grain was then readily calculated from the equation

$$k = \frac{Qd}{A\,T\,(t_1 - t_2)}$$

where:

d = thickness of the specimen

A = area under the cylinder

T = time in seconds

t_1 and t_2 = temperatures of the lower and upper surfaces of the specimen

In these studies the temperature of the boiler was maintained at or near 40° C. The moisture content was determined in the usual manner by taking a sample of the oats at the time they were tested. They were subsequently dried in an electric oven held at 100° C. until there was no further loss in weight. In order to bring the oats up to the approximate moisture content desired, a measured amount of water was added to a weighed amount of oats and flasks were shaken at intervals for three days. At the end of this time the moisture was evenly distributed. All determinations were run in duplicate and the average taken.

The oats were of Iowar variety and were grown on the Agronomy Farm the previous year.

A specific example of the calculations used is as follows:

Time—March 4, 1932

200 gm. oats + 20 gm. water Percentage moisture = 16.48

Oats placed in between the boiler and ice compartment 11:00 A.M.

Q = quantity of heat passing through; A = area; T = time; d = thickness; k = specific thermal conductivity.

$$Q = \frac{kA\ (\text{time})\ (t_1 - t_2)}{d}$$

$$k = \frac{Qd}{A\ \text{time}\ (t_1 - t_2)}$$

$$Q = \frac{3.87}{0.0836} \times 0.9164 \times 79.8 = 3385 \text{ calories}$$

$d = 0.94$ cm.

$A = 122.71$ sq. cm.

Time = 3639 seconds

Temp. diff. = 40° C.

$$\therefore k = \frac{3385 \times 0.94}{122.71 \times 3639 \times 40} = 0.0001781$$

Volume of tube no. 2 = 3.87 cc.

$$Q = \frac{3.87}{0.0836} \times 0.9164 \times 79.8 = 3385 \text{ calories}$$

I. Time

1:30—Tube 3.87 cc. inserted	40° C.	Temperature of boiler
1:45	40° C.	
2:17—37 (water at top mark of tube)	40° C.	
2:40	40° C.	
3:00	40° C.	
3:18—42 (water column at lower mark of tube)	40° C.	
Time, 1 – 1 – 05 = 3665 seconds	40° C.	

II. Time

3:29—25 (water column at top mark of tube at start)		Temperature of boiler
3:45	40° C.	
4:00	40° C.	
4:15	40° C.	
4:29—38 (water column at lower mark)	40° C.	
Time, 1 – 0 – 13 = 3613 seconds. Average temp.	40° C.	
Average time 3639 seconds		

Experimental data

Three series of experiments are reported in this paper: (1) run January 29 to April 15, 1932; (2) run October 8 to December 16, 1932; and (3) run January 6 to March 10, 1933. The first trial is reported in table I.

TABLE I

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 1, JANUARY 29 TO APRIL 15, 1932

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
January 29	9.88	0.0001527
February 12	10.51	0.0001650
February 19	11.87	0.0001668
February 26	12.34	0.0001747
March 4	16.48	0.0001830
March 11	25.23	0.0001895
March 18	27.66	0.0001910
March 25	30.32	0.0002014
April 1	34.35	0.0002149
April 8	36.82	0.0002168
April 15	38.38	0.0002220

The oats used in series 1 ranged from 9.88 to 38.38 per cent. moisture content. The thermal conductivity ranged from 0.0001527 to 0.000222. The graph (fig. 1) shows that in general the thermal conductivity increases with the moisture content.

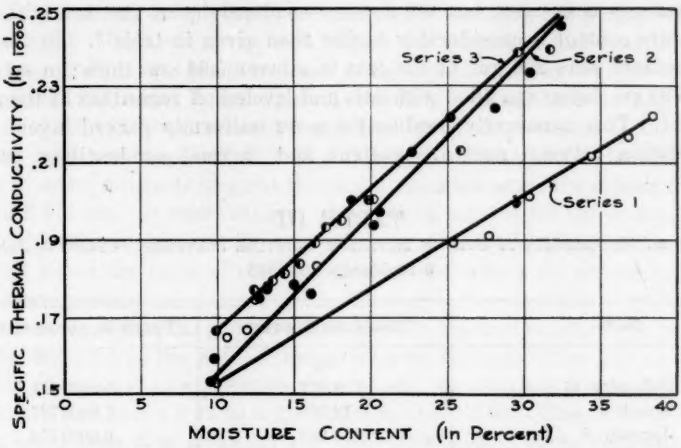


FIG. 1. Increase in specific thermal conductivity of oats with increase in percentage of moisture content.

TABLE II

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 2, OCTOBER 8 TO DECEMBER 16, 1932

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
October 8	9.54	0.000153
December 2	9.72	0.000159
November 18	12.62	0.000175
November 11	13.183	0.0001771
November 4	14.96	0.000178
October 28	16.09	0.000176
October 21	18.70	0.0002005
November 11	20.14	0.0001938
November 18	20.59	0.0001971
December 2	22.693	0.0002126
December 9	25.107	0.0002228
December 9	28.121	0.0002240
December 16	30.385	0.0002335
December 16	32.49	0.0002455

In table II and figure 1 it is found that in series 2 there is a general increased value in the thermal conductivity when the moisture content is raised. The range in moisture contents is between 9.54 and 32.49 per cent. and the thermal conductivities are found to be between 0.000153 and 0.0002455. The thermal conductivities of the air dried oats are practically

the same in both cases, but the thermal conductivity of the oats with high moisture content is considerably higher than given in table I. In the first series there were 200 gm. of the oats in a layer 0.94 cm. thick; in series 2, the 0.94 cm. layer was filled with oats and leveled off regardless of the exact weight. This apparently produced a more uniformly packed layer. The correlation between moisture content and thermal conductivity became closer.

TABLE III

THERMAL CONDUCTIVITY OF OATS OF DIFFERENT MOISTURE CONTENTS. SERIES 3, JANUARY 6 TO MARCH 10, 1933

DATE	MOISTURE CONTENT	THERMAL CONDUCTIVITY
	%	
February 17	9.877	0.0001671
March 3	12.365	0.0001767
January 6	13.202	0.0001776
February 24	13.50	0.0001701
February 3	14.37	0.0001811
January 20	15.451	0.0001840
January 20	16.324	0.0001893
February 10	17.094	0.0001935
March 10	18.073	0.0001948
February 10	19.873	0.0002000
February 17	20.254	0.0002060
February 24	25.862	0.0002134
March 3	29.640	0.0002388
March 10	31.786	0.0002393

In this series, table III and figure 1, the thermal conductivity of the air dried oats (9.877 per cent.) was 0.0001671, a little higher than in the previous two series. With a moisture content of 31.786 per cent. the thermal conductivity had arisen to 0.0002393. The graph drawn from the lowest moisture content to the highest becomes a straight line.

Discussion

In the data submitted and represented graphically in figure 1, series 2 and 3, the thermal conductivity values increase with the moisture content. In table I, weighed amounts of oats (200 gm.) were used. The tempered grain, that is, that which had had water added, had to be tamped considerably in order to get the weighed amount between the boiler and the ice compartment. This no doubt accounts for the deviation in the moisture contents from 10.51 to 12.34 per cent. In the case of the higher moisture contents the oats did not experience as much increased compression as the oats with lower moisture contents.

In series 2 and 3 the graphs are practically straight, the points are not far removed. In this case the layer of oats was tamped as uniformly as possible but no effort was made to place in this space a known weight of oats.

In table III, series 3, and figure 1, the data bearing dates on and after February 10, 1933, were secured from those where the thickness of the layer of oats used was increased to 1.48 cm. instead of 0.94 cm. as before. Instead of using a tube as large as the one in the other cases the contents were cut to 2.479 cm. It took 350 gm. of air dried oats to fill the space. The thermal conductivity results secured here are in harmony with those secured where the layer of oats was thinner and where the contents of the tube were greater.

It is evident that the moisture increases the thermal conductivity considerably, but even so the rate of heat movement through either dry or damp oats is extremely slow. STILES determined the thermal conductivity of wallboard and found it to be approximately 0.0001150. This value is somewhat lower than that given for dry oats (0.000153). Even with this difference, dry oats makes a rather effective insulating material.

In BAKKE and NOECKER'S (1) previous publication on the relation of moisture to respiration and heating, it has been pointed out that "heat pockets" are often formed. These areas are often restricted and well defined and are formed through the rapid oxidation of the grain and associated micro-organisms and the low thermal conductivity of grain even when the moisture content is high. Any method which will dissipate the heat will naturally operate against the low thermal conductivity and produce conditions which will secure a more rapid dissipation of heat. This will make for better storage conditions.

Summary

The thermal conductivity of dry oats has a value of about 0.000153, and increases directly with the moisture content. At a moisture content of 9.88 per cent., the thermal conductivity is 0.000153 and at 38.32 per cent. moisture, 0.000222 in c.g.s. units.

IOWA STATE COLLEGE
AMES, IOWA

LITERATURE CITED

1. BAKKE, A. L., and NOECKER, N. L. The relation of moisture to respiration and heating of oats. Iowa Agr. Exp. Sta. Res. Bull. 165. 1933.
2. STILES, H. Determining thermal conductivity of heat insulating materials. Chem. & Metall. Eng. **36**: 625–626. 1929.
3. ————. Improved apparatus for thermal conductivity. Chem. & Metall. Eng. **38**: 726. 1931.

SULPHUR CONTENT OF PLANTS

JOHN FRAZER

Literature review

Although it has been known for more than 70 years that sulphur, together with other elements, is essential to plant nutrition, the question of the sulphur content of plants appears to have received but scant attention. It is significant that numerous investigations are now in progress dealing with the broad question of cell growth, with special reference to sulphur content and to the function of sulphur in such growth. It may well be that more information on the subject of sulphur content and its precise rôle in plant growth will throw light upon certain obscure phases of the development of animal tissue, and eventually become a factor in the solution of the cancer problem.

Before a thorough understanding of the function of sulphur in the growth of plants is possible, more information is essential as to the quantity of this element in plants and what variations in quantity there may be in the course of plant development.

In 1911 HART and PETERSON (7) demonstrated that plant material contained much larger amounts of sulphur than had hitherto been suspected, and that its occurrence in the soil was less than that of phosphorus. PETERSON (12) showed that sulphur occurs in plant and animal tissues chiefly as protein, volatile compounds (as mustard oils), and as sulphates. He divided the sulphur of plants into four categories, (1) volatile sulphur, (2) sulphates, (3) total soluble sulphur, and (4) insoluble sulphur.

The question of the amount of sulphates in plants has been investigated by ARENDT, ULRICHT, SCHULZE, BERTHELOT and ANDRÉ, FRAPS (6), and THOMPSON (13), who found wide limits according to the amount in the soil, plant part analyzed, and the stage of growth. Other French investigators (4) experimented with sulphur and carbon bisulphide in relation to increased crop production.

MILLER (10) points out that from 2 to 100 times as much sulphur is needed for the growth of plants as was previously supposed. He divides the sulphur in plants into (1) protein sulphur (cystine), (2) volatile compounds (mustard oil, allyl and vinyl sulphides and mercaptans), and (3) sulphates. MILLER states that in some plants 65 per cent. of the sulphur is in the form of sulphates. Plants are incapable of absorbing elemental sulphur. This element is fairly evenly distributed in plants and functions as building material for the formation of protein. Furthermore it increases the root or absorbing system of the plant, the chlorophyll content, and the number of nodules of legumes.

CROCKER (5), in discussing the necessity of a sulphur carrier in artificial fertilizers, refers to a general "sulphur hunger" shown by leguminous crops, especially alfalfa, and states that in certain cases the addition of sulphur of any sort increased yields from 500 to 1000 per cent., increases of 50–100 per cent. being common. He adds that the sulphur-treated alfalfa plots of Washington and Oregon show the most striking of the responses to fertilizers. He suggests that the values for the first sulphur analyses of plants were probably low because much of the material was volatilized in the process of ashing.

Crops generally contain about equal amounts of sulphur and phosphorus, but in legumes, cabbage, mustard, potatoes, onions, and chives the sulphur content exceeds that of phosphorus. Sulphur is necessary as a building material. All plant proteins contain sulphur in cystine, and in plant proteins the percentage varies between 0.3 and 7.2 per cent. Apart from proteins, sulphur enters into other constituents of plants as building material, as in mustard oil, mercaptans, allyl and vinyl sulphides of the onion and garlic. Sulphur is also important in the formative effects in plants, and has been observed to produce a great increase in size and extent of the root system of alfalfa, as well as a marked nodule development in this plant (RITZ) and also in red clover (MILLER). In agreement, CROCKER and MILLER (10), state that sulphur assists chlorophyll development, causes increased root or absorptive systems in legumes, increased nitrogen fixing apparatus and carbohydrate manufacturing apparatus. CROCKER suggests that it may be of great significance in supplying building materials for sulphur-rich animal tissues, *e.g.*, hoofs, hair, and nails. Animal proteins, he states, are generally richer in sulphur than are plant proteins. He points out further that sulphur fertilizer ($CaSO_4$) greatly increases yields of cotton which, just before blooming time, show a content of sulphur three times as much as of phosphorus. Increases in sugar cane crops have been produced by CALVINO in Cuba, using $CaSO_4$ as fertilizer.

MARSH (9) states that the sulphur content of apple trees is of the same order as that of phosphorus. The sulphur curve is correlated directly with variations in pH. Sulphur in tomato tissue was 2½–4 times the amount of phosphorus, and the nitrogen content doubled that of sulphur. The older the tissue the higher was the sulphur content. In cases where sulphur is high, phosphorus is low and the amounts seemed to run inversely to each other, just as in the case of apple tree tissues. It has been suggested that sulphur may liberate phosphorus from the older tissues for translocation to the younger growing tissue. Sulphur seems to parallel pH. ROSA found the pH in the tomato to have the following values:

in plant tips	5.7
in fruiting region	4.8

As the same correlations were found in the apple tree tissues, there must exist some fundamental relationship between sulphur, pH, and phosphorus. Fertilizer treatments caused much variation in the amounts of phosphorus and sulphur present. The addition of acid phosphate markedly increased the sulphur and phosphorus content.

In his paper on the sulphur requirement of the red clover plant, TOTTINGHAM (**14**) states that HART and TOTTINGHAM reported increased production of dry matter by plants that had received $CaSO_4$ and Na_2SO_4 in soil culture. Red clover gave greater response to $CaSO_4$ than to Na_2SO_4. RITZ found the development of red clover bacteria to be stimulated by the sulphur from $CaSO_4$. Magnesium sulphate was found to be inferior to sodium sulphate or calcium sulphate for red clover in water or sand culture. $CaSO_4$ proved to be especially efficient. It was found that a deficiency of sulphur restricted growth by limiting the synthesis of protein.

TABLE I

SULPHUR CONTENT OF LEAVES
(PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

PLANT	SULPHUR CONTENT				
	MINIMUM	MAXIMUM	AVERAGE	NO. OF SPECIMENS	BOTANIC GARDEN, U. OF P.
	%	%	%		%
Picea	0.030	0.135	0.083	32	.....
Pinus	0.000	0.365	0.105	47	0.440
Tsuga	0.050	0.260	0.138	10	0.450
Abies	0.025	0.260	0.117	20	0.245
Taxus	0.015	0.125	0.073	15	0.350
Chamaecyparis	0.075	0.180	0.120	17	0.350
Thuja	0.030	0.110	0.051	14	.....
Juniperus	0.065	0.115	0.083	17	.....
Cryptomeria	0.105	0.105	0.105	3	0.267
Cephalotaxus	0.120	0.160	0.140	2	0.430
Cupressus	0.185	0.185	0.185	11	.....
Thujopsis	0.060	0.075	0.065	3	.....
Sciadopitys	0.335	0.410	0.370	3	.....
Rhododendron	0.100	0.275	0.161	4	0.180
Buxus	0.090	0.365	0.191	30	0.390
Mahonia	0.105	0.105	0.105	1	0.315
Hedera	0.080	0.140	0.120	4	0.490
Kalmia	0.008	0.075	0.042	2	.....
Bambusa	.....	.....	0.295	1	0.353
Aucuba	0.475	0.475	0.475		0.475
Magnolia	0.030	0.130	0.080	2	.....

It is interesting and significant to note that workers in this field are in agreement as to the influence of sulphur upon growing plants with respect to the synthesis of protein. MILLER, CROCKER, and TOTTINGHAM are apparently in entire accord on this point.

Much has been written on the question of injury to vegetation by sulphur compounds (**11**), especially by SO_2. It has been found (**16**) that very minute quantities of SO_2 acting for long periods of time are unable to disturb the normal growth of plants. Injury from smelter smoke, which has been the subject of extensive litigation, is greater immediately after irrigating the soil or after rain. The effects of such injury are more noticeable in the case of crops whose leaves are near the ground than when the leaves grow higher, and, as might be expected, young plants and their young tender parts are more seriously affected than older and hardier plants or parts.

BRIZI (**3**) found that injury to plants takes place from the exterior and not through the roots as a medium. If this be a correct assumption, the absorption of sulphur compounds from the atmosphere at once assumes added significance and importance.

The investigation here reported concerning the sulphur content of plants included particularly a study of the degree to which plants are capable of absorbing sulphur from prevailing atmospheric gases. It will be observed,

TABLE II

SEASONAL VARIATION IN SULPHUR CONTENT
(PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

PLANT	MAY 30, 1932 (OUTDOOR ARTS CO.)	NOVEMBER TO JANUARY, 1933 (MORRIS ARBORETUM)	
	%	%	
Pinus mugo	0.050	0.050	November 28, 1932
Pinus austriaca	0.115	0.045	
Pinus flexilis	0.115	0.070	
Pinus densiflora	0.095	0.075	
Chamaecyparis obtusa	0.075	0.135	January 30, 1933
Chamaecyparis crippsii	0.100	0.105	
Chamaecyparis filifera	0.110	0.080	
Chamaecyparis squarrosa	0.145	0.150	
Chamaecyparis pisifera	0.090	0.160	
Chamaecyparis plumosa	0.165	0.100	
Taxus baccata	0.090	0.060	
Taxus cuspidata	0.085	0.020	
Tsuga canadensis	0.170	0.195	
Thuja occidentalis	0.040	0.010–0.050	
Juniperus chinensis	0.090	0.100	
Juniperus argentea	.....	.....	
Juniperus pfitzeriana	0.080	0.070	
Average	0.101	0.095–0.098	

TABLE III

SULPHUR CONTENT OF LEAVES

(PERCENTAGE OF DRY WEIGHT OF PLANT MATERIAL)

	PLANT	CHESTNUT HILL, PA.
		%
Deciduous trees	*Hydrangea*	0.570
	Lilac	0.100
	Apple	0.050
	Privet	0.330
	Maple	0.225
Herbaceous herbs	Tobacco leaves	0.360
	Violet sp.	0.185
	Cauliflower stem	0.495
	Jonquil leaves	0.170
	Convallaria leaves	0.135

in the tables which are presented here, that there is considerable difference, sometimes marked, in the sulphur content of plants of the same kind when taken (a) from a locality in which the atmosphere is free from sulphurous gases; and (b) from a locality where these gases are prevalent in varying concentrations, as for example, the central section and suburbs of a large manufacturing city where smoke and gases pollute the air.

Investigation

Comparisons of analogous species from different localities were made. The analyses reveal but slight variations in sulphur content, whether the specimens were taken during the dormant season or during the period of active growth.

PROCEDURE.—Each sample was first freed from dust and any mechanically adhering matter. It was then carefully dried in a Freas electric oven at a temperature not exceeding 108° C. The leaves or needles were then separated from the woody part of the branch and bottled in glass-stoppered bottles to prevent the absorption of moisture or the effects of laboratory fumes.

For each analysis approximately 2 gm. of the material were used, the weight being taken on a delicate balance. The sample was placed in a porcelain evaporating dish, covered with distilled water, and 10 cc. of pure nitric acid (sp. gr. 1.42) added. The evaporating dish was then placed on a water bath for 2.5–3 hours until evaporated to complete dryness. To the residue were added about 40–50 cc. of boiling water and 2–3 drops of concentrated hydrochloric acid in order to dissolve any soluble matter. This was then filtered into a beaker through no. 42 Whatman's filter paper and the precipitate washed thoroughly with boiling water.

The filtrate was then brought to boiling and a slight excess of barium chloride solution added while stirring. The precipitate and solution were covered and allowed to stand for at least 12 hours, usually overnight, to permit the precipitate of barium sulphate to settle completely.

The solution was again filtered and the precipitate carefully washed with boiling water, dried, and ignited in a weighed Sillimanite porcelain crucible which was then placed in a desiccator for several hours before weighing. From the weight of the barium sulphate the weight of sulphur was calculated, and from the latter the percentage in the original sample was determined.

Conclusion

The results indicate that certain species of coniferous plants and others obtained from localities where there is distinct atmospheric contamination from factories, chimneys, etc. contain several times as much sulphur as corresponding species from a region where the influence of such atmospheric pollution is nil, or so slight as to be negligible.

To the Director of the Botanic Garden and of the Morris Arboretum of the University of Pennsylvania, from which sources numerous specimens were supplied for this investigation, the writer expresses his grateful appreciation.

UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PA.

LITERATURE CITED

1. BAKKE, A. L. The effect of city smoke on vegetation. Iowa Agr. Exp. Sta. Bull. 145. 1913.
2. BOULLANGER, E. Action du soufre en fleur sur la végétation. Compt. Rend. Acad. Sci. Paris **154**: 369–370. 1912.
3. BRIZI, UGO. Sulle alterazione prodotte alle piante coltivate dalle principali emanazione gasose degli stabilimente industriale. Staz. Sper. Agr. Ital. **36**: 279–384. 1903.
4. CHANCRIN, E., and DESRIOT, A. Influence du soufre sur la végétation des pommes de terre et des betteraves. Jour. Agr. Prat. **76**: (n. s. **23**:) 365–367. 1912.
5. CROCKER, WILLIAM. The necessity of sulphur carriers in artificial fertilizers. Jour. Amer. Soc. Agron. **15**: 129–141. 1923.
6. FRAPS, G. S. Determination of sulphates in plants. North Carolina Agr. Exp. Sta. 26th Ann. Rept. Pp. 69–71. 1903.
7. HART, E. B., and PETERSON, W. H. Sulphur requirements of farm crops in relation to the soil and air supply. Wisconsin Agr. Exp.

Sta. Res. Bull. 14. 1911. (See also Jour. Amer. Chem. Soc. **33**: 549–564. 1911.)

8. Maizières, Mons. Le soufre comme nouvelle matière fertilisante. L'Engrais **36**: 684–685. 1911.
9. Marsh, R. S. Preliminary studies on the sulfur content of the tomato. Amer. Soc. Hort. Sci. Proc. **19**: 83–84. 1922.
10. Miller, E. C. Plant physiology. Pp. 257–259. McGraw-Hill Book Co., New York. 1931.
11. Monnett, O., Perrot, G. St. J., and Clark, H. W. Smoke abatement investigation at Salt Lake City, Utah. U. S. Dept. Commerce, Bur. Mines Bull. 254. 1926.
12. Peterson, W. H. Forms of sulfur in plant materials and their variation with the soil supply. Jour. Amer. Chem. Soc. **36**: 1290–1300. 1914.
13. Thompson, Alice R. The determination of sulfur and chlorine in the rice plant. Jour. Amer. Chem. Soc. **35**: 1628–1634. 1913.
14. Tottingham, W. E. The sulfur requirement of the red clover plant. Jour. Biol. Chem. **36**: 429–438. 1918.
15. Vercier, J. Experiences sur l'action fertilisante du soufre. Prog. Agr. et Vitic. **58**: 695–697. 1912.
16. Widtsoe, J. A. The relation of smelter smoke to Utah agriculture. Utah Agr. Exp. Sta. Bull. 88. 147–179. 1903.

BIOCHEMICAL PHASES OF OLEORESIN PRODUCTION[1]

ELOISE GERRY AND J. A. HALL

(WITH FOUR FIGURES)

The key position of naval stores production in the profitable utilization of the more than 50,000,000 acres of longleaf and slash pine lands of the southeastern United States puts a premium upon exact and comprehensive knowledge of the biochemical facts which underlie successful oleoresin production. Oleoresin, or gum, the source of naval stores, exudes from cuts on living pines, from their earliest seedling stage. A cut into a young sapling, for example, illustrates the abundance of the exudation. The exudate is not the sap of the tree in the ordinary sense of the term, however, nor does it come from the ordinary sap-conducting tissues as does the watery solution of the exuding maple sap.

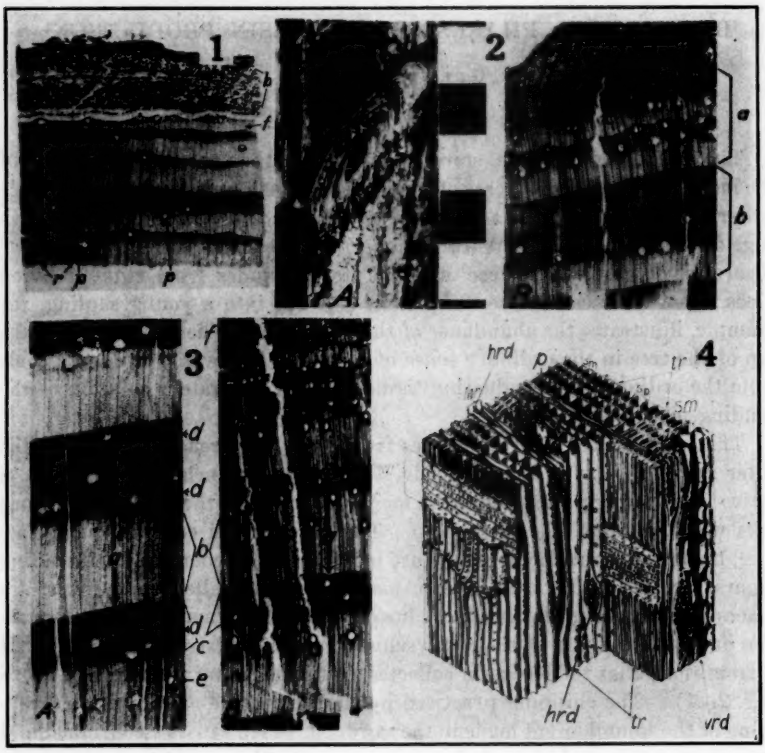
The exudation of oleoresin comes from areas of parenchyma cells in the outer sapwood next to the bark (fig. 1). These parenchyma cells contain active functioning protoplasm for a longer period than the associated fibrous cells which conduct the watery sap. The parenchyma cells also manufacture, transform, translocate, and store many different compounds, including sugars, tannins, and starch. Resin passages, the so-called resin ducts, are spaces which arise in the midst of clusters of parenchyma cells by a separation or splitting apart of the cells (schizogenous not lysigenous origin). It is from these that the oleoresin collected in turpentining wells out in drops (fig. 2, *A*). The chipping practiced by the turpentine operator is a freshening of the wound or cut made in the sapwood, which causes a continued and even augmented exudation of oleoresin.

Resin passages are an outstanding characteristic of all pine wood, but they reach an optimum development in slash (*Pinus caribaea*) and longleaf (*P. palustris*) pines. They extend both vertically and horizontally in the tree. The two systems frequently connect with each other and with the rays (also parenchymatous in nature) at different levels, thus forming an extensively anastomosing system which is also connected with the parenchyma tissues of the phloem or inner bark through which compounds elaborated in the leaves are translocated (fig. 1).

Resin passages are more numerous than is often realized. Recent microscopical investigations at the Forest Products Laboratory have shown (7)

[1] Contribution from the Forest Products Laboratory, maintained at Madison, Wisconsin, in cooperation with the University of Wisconsin.

This paper was presented before the Division of Agricultural & Food Chemistry at the annual meeting of the American Chemical Society, St. Petersburg, Florida, March, 1934.



FIGS. 1–4. **Fig. 1:** Microscopical view of cross section of pine wood showing vertical resin passages (*p*) at center of groups of parenchyma cells; rays (*r*), including a wide ray with a horizontal resin passage connecting a vertical resin passage with the inner bark or phloem (*b*) rich in stored material; and the wood tracheids or fibers (*f*) which function in water conduction. **Fig. 2:** *A*, heavy exudation next bark obtained from the increased number of wound-induced resin passages; *B*, wood above the face or wound on a turpentined pine; *b*, normal wood formed before turpentining; *a*, two years of growth during turpentining showing great increase in number of resin passages. **Fig. 3:** *A*, cross section of normal southern pine wood (slash pine); *a*, portion of one annual growth ring; *b*, springwood; *d*, summerwood; *e*, resin passages; *B*, wood formed by same tree over face during turpentining, containing greatly increased number of resin passages; *a*, portion of one annual growth ring. **Fig. 4:** Diagrammatic drawing of pine wood structure showing horizontal resin passages (*hrd*) and vertical resin passages (*vrd*) and the connections frequently found between them. Also springwood fibers (*sp*), summerwood fibers (*sm*), and rays (*wr*, *fwr*).

that in slash pine on the average more than 450 horizontal resin passages were present per square inch of tangential surface (fig. 4). The number present in various specimens ranged from about 250 to 800 per square inch. The exudation from these horizontal resin passages is more directly utilized in the French method of turpentining than in the American, because a relatively greater area of tangential surface is regularly freshened owing to the shape of the French wound or face.

The vertical resin passages, which are much larger than the horizontal passages, number about 200 per square inch of cross section in normal wood. This number ranges from about 70 to 400 per square inch. In wood formed above the face during turpentining the number of vertical resin passages may be increased in varying amounts up to more than tenfold the normal number (fig. 3). The induced increase of resiniferous tissues by wounding is of considerable importance since a large proportion of the yield obtained after the first year of turpentining comes from it (fig. 2, *A*). The effect of the wound may extend vertically for 10 to 20 feet above the face and is clearly registered in the structure of the wood formed above the face. The wound response is limited to a few inches circumferentially, however, although characteristic traumatic structures are apparent in the rolls of healing tissue formed at the sides of the face. They often offer a rich source of oleoresin when trees are back-cupped during successive workings.

Investigations other than chemical have dealt to date with anatomical and physiological studies of the normal character of resiniferous tissue in virgin and second-growth trees, and with the responses induced by different types of wounding. The work now in progress is a joint attack, using in addition cytological, microchemical, and biochemical techniques to determine the character and sequence of changes in the compounds which underlie oleoresin formation.

Oleoresins are biological materials, products of the living protoplasm, and therefore referable to the original products of photosynthesis for their ultimate chemical derivation. It must be said that here, however, as with most other complex substances of vegetable origin, not one step is yet known with certainty in the complex processes by which the pine tree elaborates oleoresin. But if empirical experimentation in forest management and turpentining is to be firmly grounded, all possible information must be obtained concerning the biochemistry of oleoresin formation.

Although technologically pine oleoresin has usually been considered as merely a solution of resin acids in a mixture of terpenes, such a concept does not suffice in an investigation of this kind. As a product of vital processes, oleoresin might be expected to contain small quantities of materials not yet completely elaborated, the careful examination of which may throw considerable light on the processes immediately preceding the excretion of the oleo-

resinous mixture. A part of this investigation therefore involves the thorough examination of substances in the oleoresin other than terpenes and resin acids, particularly such materials as are soluble in water.

The place of excretion of the completed oleoresin is known, as has been indicated (fig. 2), although the mechanism of the process is unknown. Similarly the processes by which assumed complex oleoresin precursors are translocated are unknown, as well as the seat of formation within the tree of these assumed complexes. A second phase of this investigation, therefore, involves the chemical examination of the tissues of the pine which are apparently concerned in oleoresin formation. A definite search is being made for substances which may be defined as oleoresin in process of formation.

Such a concept is by no means original, although comparatively little evidence has hitherto been brought forward in support of it. KÖHLER (**8**) found certain resinous secretions in *Picea excelsa,* which were subject to rapid and spontaneous change upon exposure to the air. He postulated an aldehydic or camphoraceous precursor, $C_{10}H_{16}O$, which was secreted as such, and changed to the mixture of resin acids and terpenes outside of the living cells.

DUPONT (**4**) developed an ingenious formulation, *viz.,*

$$3\ C_{10}H_{16}O = C_{10}H_{16} + C_{20}H_{30}O_2 + H_2O.$$

The elaboration of this equation explains very satisfactorily the proportions of resin acids and terpenes found in the oleoresins from *Pinus maritima* and *P. halepensis,* in which the ratio of rosin to turpentine approximates closely the 69 : 31 ratio required by DUPONT'S equation. However, numerous pines do not approximate this composition in their oleoresins; slash and longleaf oleoresins run more nearly a ratio of 80 : 20 (**1**). It is also true that there is appreciable variation in turpentine content between individual trees. Further, it must be considered that any such mechanism must also explain the fact of the existence of n-heptane as the volatile ingredient of oleoresins from *P. jeffreyi* and *P. sabiniana,* as well as the large proportions of sesquiterpene in the oleoresin of *P. longifolia.* In short, although the assumption of some common precursor of volatile and non-volatile components of oleoresin has a logical basis, it does not seem that so simple a balance as indicated by DUPONT can explain the facts of oleoresin composition throughout the genus *Pinus.*

DEVAUX and BARGUES (**3**) showed that the oleoresinous materials were not entirely localized in the resin passages of the wood and in the cells bordering them. The living parenchyma cells of the wood and phloem are connected with the resin passages through the rays (fig. 2) which allow translocation of the materials which are concerned in oleoresin production. Noteworthy is the statement of DEVAUX and BARGUES that the secretion is acidic

only in the canals and generally neutral in the living cells. The change is said to occur when the resin reaches the canal, although it may occur prematurely.

GLICHITCH and NAVES (**5**) call attention to the difference in sesquiterpene content of ylang-ylang oils obtained by steam distillation and petroleum ether extraction. The very low content of sesquiterpenes in the latter leads them to suggest that the sesquiterpenes may arise during the distillation process from substances insoluble in petroleum ether and non-volatile with steam.

There is further the evidence of BRIDEL (**2**) concerning the existence of glucosides of geraniol, which gives a clue to the possible nature of the mechanism by which substances insoluble in cell sap are held in solution by means of glucosidal combinations.

A recent theoretical study (**6**) showed the probable derivation of the terpenes from underlying hydroxylated bodies. A similar type of generalization doubtless holds for the resin acids although sufficient examples of known structure are not yet available to warrant their classification. Such assumptions seem more warranted than the well known isoprene polymerization in that nothing has been observed indicating the existence of isoprene in nature, nor has it been formed *in vitro* by means analogous to those of biological reactions.

From these considerations, the search for oleoresin precursors both in the oleoresin and in the tissues of the tree has been primarily for glucosidal materials, water-soluble and resolvable into oleoresinous constituents and sugars. The difficulties of this work are apparent, since it involves the isolation of very small quantities of delicate compounds from very large quantities of starting material by means sufficiently gentle to avoid decomposition. While no single phase of the problem may be regarded as completed, considerable progress has been made which is briefly reported here.

The oleoresin itself contains small quantities of highly complex glucosidal material which is soluble in water and can be obtained by treatment of the oleoresin with water. The original clear, light, aqueous extract soon deposits a black, amorphous precipitate which is now insoluble in water but soluble in alcohol. This substance is easily hydrolyzed, yielding a large proportion of acidic bodies of a resinous nature. While these substances cannot yet be identified, since they have been available to the extent of only a few grams, their color reactions are those of the acids of rosin, and it is extremely probable that they belong to this class of substances.

The solution from which this dark precipitate has been obtained still contains a considerable quantity of material in solution. By extraction with ether, a substance is obtained which is instantly hydrolyzed by very dilute acid. It yields a resin acid, a volatile oil, and a water-soluble body. The

last may be broken up further into a sugar and a resin acid. The sugar is remarkable in that it reduces Fehling's solution in the cold, and reacts with phenylhydrazine very quickly in the cold, yet it is not an uronic acid. It is highly reactive and tends to polymerize to unworkable complexes on very slight provocation.

Complete interpretation of these results is still far distant, and must await considerably more material and effort. However, they suggest very definitely the presence in the oleoresinous exudate of glucosidal complexes of resin acids, volatile oil, and reactive sugars, very easily broken up and very sensitive to slight changes in environment.

Investigation of slash pine tissues has so far been confined to the phloem, since, as was pointed out, this seems to constitute a rich reserve tissue (fig. 2), physiologically closely related to that immediately concerned with production of the oleoresin in the resin passages of the wood or xylem. While a rather complete phytochemical survey of this material has been made, it will suffice in this connection to point out only the occurrence in the phloem of glucosidal complexes of exactly similar nature to those obtained from the oleoresin itself; that is, a glucoside soluble in both water and ether, yielding to very mild acidic hydrolysis in the cold, resin acid, volatile oil, and the same resin acid-sugar complex as obtained from the oleoresin.

It is regretted that further data on the constitution of these substances cannot be given at this time, but their extremely complex nature makes necessary the preparation of further quantities of material for more complete investigation. It is believed, however, that the attack thus far has been justified and has yielded considerable chemical substantiation of the anatomical evidence of functional connection between resiniferous tissues of both phloem and xylem, and thrown considerable light on the processes preceding the excretion of oleoresin at the wounded surface.

At present the picture presented is of a large complex of resin acids and terpenes combined with sugars, the whole being thus soluble in the elaborated sap of the parenchyma cells and translocated as such to the neighborhood of the area of excretion, where it is resolved by a mechanism still obscure.

In conclusion, it may be pointed out that the demonstrated presence of these highly reactive, water-soluble bodies in the oleoresin contributes in no small degree to the variations in color of manufactured rosins, and is thus of considerable technological importance.

FOREST PRODUCTS LABORATORY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

LITERATURE CITED

1. BLACK, A. P., and THRONSON, S. M. Oleoresin from individual trees of slash and longleaf pine. Ind. & Eng. Chem. **26**: 66–69. 1934.

2. Bridel, M. Sur la présence d'un glucoside à essence dans les tiges foliées et les racines du *Sedum telephium* L. Jour. Pharm. Chim. (7) **26**: 289–298. 1922.
3. Devaux, H., and Bargues, A. Le système sécréteur oléorèsineux du pin maritime. Proc.-verb. Séances Soc. Phys. Nat., Bordeaux 1925–1926: 174–182. Bull. Inst. du Pin 131–135. 1927.
4. Dupont, G. Hypothèse sur les relations d'origine entre les terpènes et les acides cristallisés constituant les résines des coniferes. Compt. Rend. Acad. Sci. Paris **178**: 1560–1562. 1924.
5. Glichitch, L. S., and Naves, Y. R. Sur la composition de l'essence d'ylang-ylang. Les Parfums de France **10**: 7–13; 36–40. 1932.
6. Hall, J. A. Biogenetics in the terpene series. Chem. Rev. **13**: 479–499. 1934.
7. Hobert, V. C. Number and distribution of resin passages in slash pine. Forest Worker **8**: 8–9. 1932.
8. Köhler, J. Beiträge zur Kenntniss des Vorkommens und der Bildungsweise der Harzsäuren. Jour. prakt. Chem. (2) **85**: 523. 1912.

SOME EFFECTS OF THE PHOTOPERIOD ON DEVELOPMENT OF *IMPATIENS BALSAMEA*[1]

J. PERRY AUSTIN

(WITH TWO FIGURES)

Introduction

In 1920 GARNER and ALLARD (1) discussed the influence of length of the daily light period on flowering. A later paper by the same investigators (2) recorded further observations of its effect on flowering and other phases of plant development.

GARNER and ALLARD (1) proposed two terms, *photoperiodism,* "to designate the response of an organism to the relative length of day and night," and *photoperiod,* "to designate the favorable length of day [for flowering] for each organism." *Photoperiodism* appears frequently in the literature on the subject. Shortly after the term *photoperiod* was proposed, however, it was observed that the length of the daily light period also influences phases of plant development other than reproduction. The term thus became unserviceable and apparently has never been used since. To make it useful and to give it a logical relation to the term *photoperiodism,* it is therefore suggested that *photoperiod* be used to designate the daily period of illumination under the influence of which an organism is developing. It is so used in this paper.

The experimental work here reported was carried on from the middle of March to the first of August, 1932. Plants of *Impatiens balsamea,* variety White Perfection, were grown in pots in the greenhouse and were subjected to photoperiods of 8, 16, and 20 hours, and normal. The normal photoperiod (sunrise to sunset) during the experiment ranged from 12 hours at the beginning to approximately 15 hours at the end. The plants were all kept in one section of the greenhouse during the day to secure as nearly uniform conditions of light intensity and temperature as possible. Eight hours after sunrise one lot of plants was removed to a ventilated darkroom until after dark when they were returned to their original places. Late in the afternoon two lots were removed to other sections of the greenhouse where they received electric illumination from sunset until 8 hours and 4 hours before sunrise, giving 16- and 20-hour photoperiods respectively. The intensity of the artificial light was approximately 125 foot-candles at the apices of the plants, as measured by the MacBeth illuminometer. The plants receiving artificial light were returned to their original places early in the morning. During the cooler weather at the beginning of the experiment the tempera-

[1] Paper from the Department of Botany, University of Michigan, no. 467.

ture was kept between 18° and 23° C., day and night; during the late spring and summer, however, the temperature often rose to 30°–35° C. about noon. The temperatures of the darkroom and auxiliary sections were approximately the same as in the main compartment.

Investigation

In general, the plants subjected to the 8-hour photoperiod appeared much less vigorous vegetatively than did those under the longer photoperiods. The flowers and seed pods were about half the size of the others. The seeds produced by the former were of normal size, but there were fewer seeds per pod than under the longer photoperiods.

Effect of length of photoperiod on leaf area

At intervals during the experiment, certain leaves were removed from groups of ten plants each from those growing under the various photoperiods. Blue-prints were made of the leaves and their areas were measured with a planimeter. The average areas are tabulated in table I. The "first leaf" is the lower of the two plumule leaves.

TABLE I

Area in square cm. of certain leaves from plants of *Impatiens balsamea* grown under various photoperiods

Age of plants	Length of photoperiod			
	8 hours	Normal	16 hours	20 hours
	sq. cm.	*sq. cm.*	*sq. cm.*	*sq. cm.*
		Cotyledons		
14 days	2.1 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	3.2 ± 0.1
27 days	1.8 ± 0.1*	2.8 ± 0.3*	3.1 ± 0.3	3.8 ± 0.1
		Plumule leaves		
14 days	1.4 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	3.3 ± 0.1
27 days	3.0 ± 0.1	6.9 ± 0.2	7.6 ± 0.3	7.7 ± 0.3
		First leaf		
41 days	3.4 ± 0.2	6.3 ± 0.4	6.4 ± 0.3	6.1 ± 0.2
55 days	3.6 ± 0.1	6.2 ± 0.4*	6.4 ± 0.4	5.7 ± 0.2*
		Fifth leaf		
41 days	5.2 ± 0.4	10.2 ± 0.4	11.2 ± 0.5	8.3 ± 0.6
55 days	4.9 ± 0.3*	9.8 ± 0.3*	12.5 ± 0.6	9.8 ± 0.4
		Tenth leaf		
55 days	.....	8.1 ± 0.5	9.7 ± 0.7	9.8 ± 0.7

* The areas given are averages of leaves from ten plants of a particular age. The areas given for the leaves from plants of different ages are based on averages of different plants; hence the apparent decrease in area in the starred instances.

From the data it is clear that the differences between the areas of leaves developed under the three longer photoperiods are not sufficiently great to be significant. On the other hand, in every instance the leaves developed under the 8-hour photoperiod are markedly smaller than the others.

Effect of length of photoperiod on budding and flowering

The plants were examined daily, and the dates when the flower buds first became visible and when the first flower opened on each plant were recorded. The data on age at budding are tabulated in table II. The columns headed "frequency" indicate the number of plants on which flower buds appeared at the age in question.

TABLE II

Age at which flower buds appeared on plants of *Impatiens balsamea* grown under various photoperiods. Columns headed "frequency" indicate number of plants on which buds appeared at the age in question

Length of photoperiod							
8 hours		Normal		16 hours		20 hours	
Age	Frequency	Age	Frequency	Age	Frequency	Age	Frequency
19	1	20	3	56	2	55	1
20	9	21	4	57	5	56	3
21	15	22	24	58	6	57	1
22	38	23	33	59	18	58	11
23	12	24	12	60	3	59	9
24	1	25	4	63	1	60	3
25	1	26	1	65	3	61	1
27	1			66	1	66	1
				68	1	68	2
				70	1	70	2
				72	2	76	2
				76	2	78	3
				78	5	87	2
						90	1
Average age at budding						91	1
21.8 ± 0.1		22.8 ± 0.1		62.7 ± 0.7		64.5 ± 0.95	

The average ages at budding under the 8-hour and under the normal photoperiods do not differ significantly. The same is true of the average ages at budding under the 16- and 20-hour photoperiods, budding occurring considerably later than under the two shorter photoperiods.

Table III contains the data on age at flowering. The average ages at flowering under the various photoperiods bear the same relation to one another as do the ages at budding. Furthermore, a comparison between the average ages at budding and at flowering (table IV) shows that the number

TABLE III

AGE AT WHICH FIRST FLOWER OPENED ON PLANTS OF *IMPATIENS BALSAMEA* GROWN UNDER VARIOUS PHOTOPERIODS

LENGTH OF PHOTOPERIOD							
8 HOURS		NORMAL		16 HOURS		20 HOURS	
AGE	FREQUENCY	AGE	FREQUENCY	AGE	FREQUENCY	AGE	FREQUENCY
43	3	43	1	62	1	77	1
44	1	44	1	67	1	84	4
45	4	46	4	75	1	86	2
46	5	47	7	85	3	87	11
47	9	48	7	87	14	90	2
48	3	49	15	89	2	91	4
49	8	50	10	91	4	92	1
50	6	51	6	95	1	93	1
51	4	52	2	97	1	99	2
52	1	53	3	101	1	100	1
53	4	54	1	103	1	106	1
54	5	57	2	106	1	108	1
55	1	59	1	109	1	115	1
56	2	61	1	110	1	125	1
58	1			111	1	132	1
				112	1	136	1
				115	1		
				130	1		
Average age at flowering							
49.2 ± 0.3		49.6 ± 0.3		92.0 ± 1.4		92.8 ± 1.6	

of days from budding to flowering is practically the same under all four photoperiods. This indicates that in *Impatiens balsamea* the effect of the length of the photoperiod with respect to the reproductive phase of development is on the initiation of the flower buds, the development of the flower from the bud being unaffected.

TABLE IV

RELATION BETWEEN AVERAGE AGE AT FLOWERING AND AT BUDDING

	LENGTH OF PHOTOPERIOD			
	8 HOURS	NORMAL	16 HOURS	20 HOURS
Age at budding (in days)	21.8 ± 0.1	22.8 ± 0.1	62.7 ± 0.7	64.5 ± 0.95
Age at flowering (in days)	49.2 ± 0.3	49.6 ± 0.3	92.0 ± 1.4	92.8 ± 1.6
Days from budding to flowering	27.4 ± 0.3	26.8 ± 0.3	29.3 ± 1.6	28.3 ± 1.9

The facts that the average age at budding under the 8-hour photoperiod is the same as under the normal, and that the average age at budding under the 16-hour photoperiod is the same as under the 20-hour, suggest that somewhere between 12 and 16 hours there must be a critical length of photoperiod.

Effect of length of photoperiod on development of main axis

The height of the main axis was measured at approximately weekly intervals. The average heights at definite ages are tabulated in table V together with the number of plants upon which each average is based. The data are presented graphically in figure 1. The average ages at budding and at

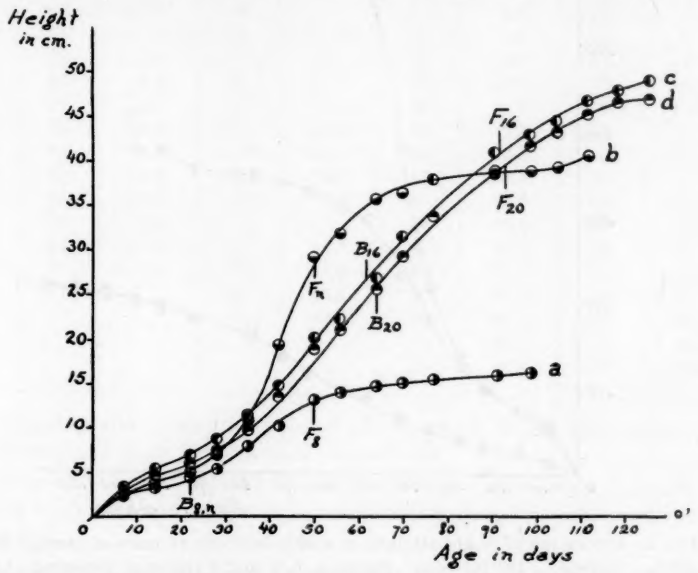


Fig. 1. Relation between length of photoperiod and development of main axis in terms of average height at weekly intervals. Curves *a*, *b*, *c*, and *d* represent average heights of plants grown under 8-hour, normal, 16-hour, and 20-hour photoperiods respectively. $B_{8,\ n,\ 16,\ 20}$ indicate time of budding (average) under respective photoperiods. $F_{8,\ n,\ 16,\ 20}$ indicate time of flowering (average) under the corresponding photoperiods.

flowering are also indicated. The height attained is greater under successively longer photoperiods up to 16 hours; a 20-hour photoperiod resulted in slightly but consistently shorter plants than those under the 16-hour photoperiod, which must therefore be approximately the optimum for development of the main axis.

A consideration of the average height attained at various ages relative to the height at budding (fig. 2) emphasizes the fact that at the time the

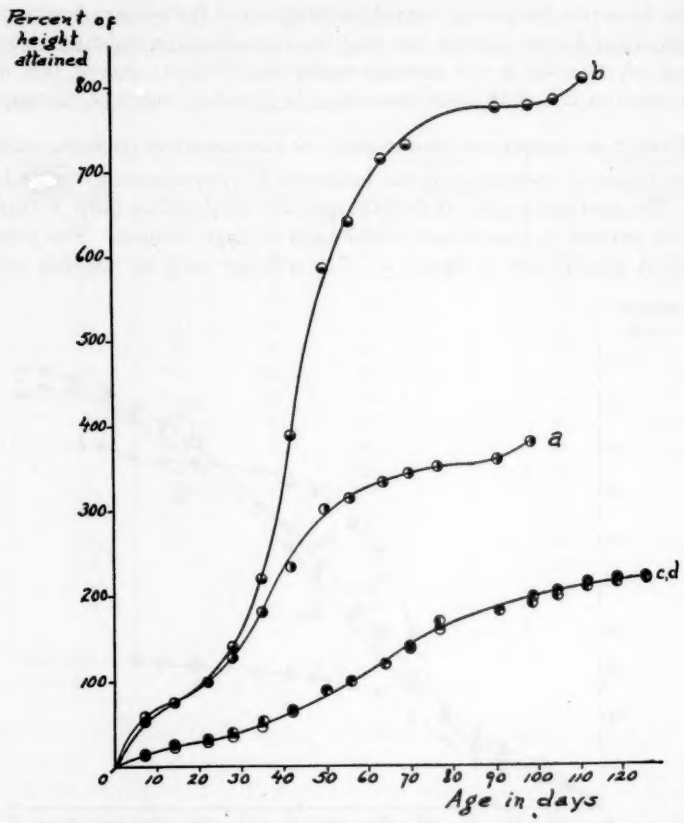


FIG. 2. Percentage of height attained at weekly intervals on basis of average height at budding (average) = 100 per cent. Curves *a*, *b*, *c*, and *d* represent percentage height at budding attained by plants grown under 8-hour, normal, 16-hour, and 20-hour photoperiods respectively.

flower buds appeared the plants grown under the 8-hour photoperiod had attained approximately one-fourth of their total height; those under the normal photoperiod, one-eighth; and those under the 16-hour and 20-hour photoperiods, one-half of their total height. This seems to indicate a considerable degree of independence between the effect of the length of the photoperiod on stem development and its effect on budding.

Discussion

With respect to the effect of the length of the photoperiod on flowering, the preceding data indicate that *Impatiens balsamea* is intermediate between

TABLE V

AVERAGE HEIGHT, AT WEEKLY INTERVALS, OF PLANTS OF *IMPATIENS BALSAMEA* GROWN UNDER VARIOUS PHOTOPERIODS

AGE	LENGTH OF PHOTOPERIOD							
	8 HOURS		NORMAL		16 HOURS		20 HOURS	
	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT	NO. OF PLANTS	AVERAGE HEIGHT
days		*cm.*		*cm.*		*cm.*		*cm.*
7	92	2.6	94	2.6	93	3.3	89	2.9
14	90	3.3	91	3.8	92	5.5	87	4.7
22	76	4.4	79	5.0	80	7.0	74	6.1
28	68	5.6	71	7.1	72	8.9	65	7.4
35	67	8.0	71	11.0	72	11.4	65	9.7
42	57	10.3	61	19.5	62	14.8	55	13.5
50	55	13.3	59	29.4	60	20.2	53	19.0
56	47	13.9	51	32.1	52	22.3	45	21.1
64	41	14.8	46	35.8	47	26.8	40	25.6
70	46	15.2	51	36.6	50	31.4	45	29.2
77	25	15.5	.....	.....	29	38.1	28	33.8
91	36	15.9	40	38.8	40	40.9	40	38.4
99	26	16.3	29	38.9	30	42.9	30	41.7
105			28	39.2	30	44.4	30	43.2
112			25	40.5	29	46.7	30	45.1
119					17	47.8	15	46.4
126					13	48.8	15	46.8

the long-day and short-day types of plants which flower only under relatively long and short photoperiods respectively.

Although the data suggest a number of lines of speculation, it seems best to defer extensive interpretations until similar data from other plants are available.

Summary

Plants of *Impatiens balsamea* were grown under 8-hour, normal, 16-hour, and 20-hour photoperiods and data obtained on leaf area, age at budding and flowering, and development of the main axis. The data bring out the following facts in regard to the response of this species to the length of the photoperiod:

1. The leaf area is approximately the same under the three longer photoperiods but is much less under the shortest one.

2. The effect of the length of the photoperiod on the time of flowering is primarily through its effect on the initiation of flower buds.

3. There must be a critical length of photoperiod, between 12 and 16 hours, with respect to the age at which flower buds are initiated.

4. The optimum length of photoperiod for stem development is apparently about 16 hours.

5. The effect of the length of the photoperiod on budding seems to be independent of the effect on stem development.

This work was done under the direction of Dr. F. G. GUSTAFSON.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

LITERATURE CITED

1. GARNER, W. W., and ALLARD, H. A. Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour Agr. Res. **18**: 553–606. 1920.
2. ————, and ————. Further studies in photoperiodism, the response of the plant to relative length of day and night. Jour. Agr. Res. **23**: 871–920. 1923.

FACTORS RENDERING THE PLASMOLYTIC METHOD INAPPLICABLE IN THE ESTIMATION OF OSMOTIC VALUES OF PLANT CELLS[1]

ELIZABETH C. M. ERNEST

(WITH TWO FIGURES)

In 1884 DE VRIES (**10**) introduced a new method, that of determining the osmotic value of plant cells by immersion in solutions of known concentrations of cane sugar or potassium nitrate. He determined the concentration of each solute which would bring about *plasmolysis* or the withdrawal of the protoplasm from the walls of the cells. He reasoned that all solutions inducing the same minimum degree of visible plasmolysis of cells must be of equal strength. Various aspects of the phenomenon of plasmolysis have been elaborately studied since then by DE VRIES (**11**), FITTING (**4**), HANNIG (**5**), ILJIN (**8**), HÖFLER (**6, 7**), URSPRUNG and BLUM (**9**), BECK (**1, 2**), WEBER (**12, 13**), and many others. These workers have interpreted the results obtained by this method as furnishing adequate data for the estimation of the osmotic value of the cell sap.

Objection may be raised to this interpretation on two grounds. In the first place, the material used by DE VRIES, and by most of his followers, cannot be considered to have been in normal, uninjured condition. Thin sections were prepared for microscopic examination by sectioning, thus subjecting the cells to the deleterious osmotic and other actions of sap exuded from adjacent ruptured cells. That this influence may be important has been pointed out elsewhere (**3**). Moreover, the phenomenon commonly designated as plasmolysis, although usually regarded as purely osmotic in nature, may be induced by several other causes. Mechanical injury induced by pressure brings about a contraction of the protoplasm which in its early stages resembles plasmolysis and which is often reversible if the injury is slight. It passes through a plasmolysis-like stage to an irreversible granulation if the pressure is considerable or of long duration. Strong light (**13**) and high temperature likewise cause retraction of the protoplasm from the cell walls, and light and temperature conditions which are not markedly injurious may influence the time required for plasmolysis.

In the second place, the assumption made by URSPRUNG and BLUM (**9**), and with few exceptions accepted without experimental proof by subsequent workers, that the cell contents and applied solution come into equilibrium within 20–30 minutes, can be shown to be untenable both on theoretical

[1] This work was done at the Imperial College of Science and Technology, London. The writer wishes to express her thanks to Professor V. H. BLACKMAN for his interest and advice.

grounds and on the basis of experiments such as those to be presented here. An uninjured protoplast, when immersed in a hypertonic solution, will lose water to the solution at a rate proportional to the difference between the osmotic pressure of the external solution and that of the cell contents. The rate of water loss from the cell will decrease as the internal osmotic pressure, owing to concentration of the sap by loss of water, increases to a value nearer that of the outer solution. A protoplast, immersed in any given solution and showing at any arbitrarily chosen moment incipient plasmolysis, may therefore subsequently contract further and may even reach the completely "balled" stage if observed for a long enough time. *A persistent incipient plasmolysis is an unattainable state.* The factors which HÖFLER (7, p. 301) enumerates as determining the decrease in size of a protoplast in a given solution, namely, conditions of approach of the solution to the cell, permeability of the cell wall to the dissolved substance in the plasmolyticum, passage of water through the plasma from the cell sap, etc., may also affect to some extent the plasmolysis time (*i.e.*, the time before the first appearance of plasmolysis) of comparable cells in different solutions. Persistent plasmolysis is not only unattainable, but it would also appear useless to assign an arbitrary time limit after which approximate equilibrium between the cells and the plasmolyticum should be assumed, and meaningless to give plasmolysis results in terms of both concentration and time.

In the present work an attempt has been made to rule out the first objection, and to evaluate the significance of the second. Sections were replaced by "strip" preparations, as discussed elsewhere (3). Cells which were uninjured and which had not been in contact with injured cells were obtained in the following manner. A sharp scalpel was inserted obliquely into the surface tissues of the leaf or other organ to be studied and the epidermis torn away. This was in many cases accompanied by a single stratum of mesophyll cells which had been subjected to no injurious influences other than the slight strain involved in separating them from the subjacent tissues. Only cells situated at least three cells distant from the periphery of the preparations were examined, since the behavior of cells at the periphery is always anomalous. Strips prepared in this way were cut into convenient lengths, immersed in sugar solutions of graded concentration, and the time elapsing between immersion and the first appearance of plasmolysis recorded.

The validity of the plasmolytic method as a measure of the osmotic value of the cell sap, assuming that uninjured cells have been used and mechanical and pseudo-plasmolytic factors are ruled out, rests on the assumption that a relation exists between concentration of plasmolyticum and time required for completion of plasmolysis such that a definite concentration can be determined above which plasmolysis occurs within a brief

time while below this concentration plasmolysis will not occur even after a long period. Such a condition is illustrated in the ideal curve of figure 1, *A*. Here plasmolysis would not occur after any length of time in a plasmolyticum of 0.450 M concentrations, while at all concentrations above 0.470 M plasmolysis would be evident within 20 minutes after immersion. Figures 1, *B* and 2 illustrate the experimental data for the seven plants tested. The ideal curve is rather closely approximated by the experimental

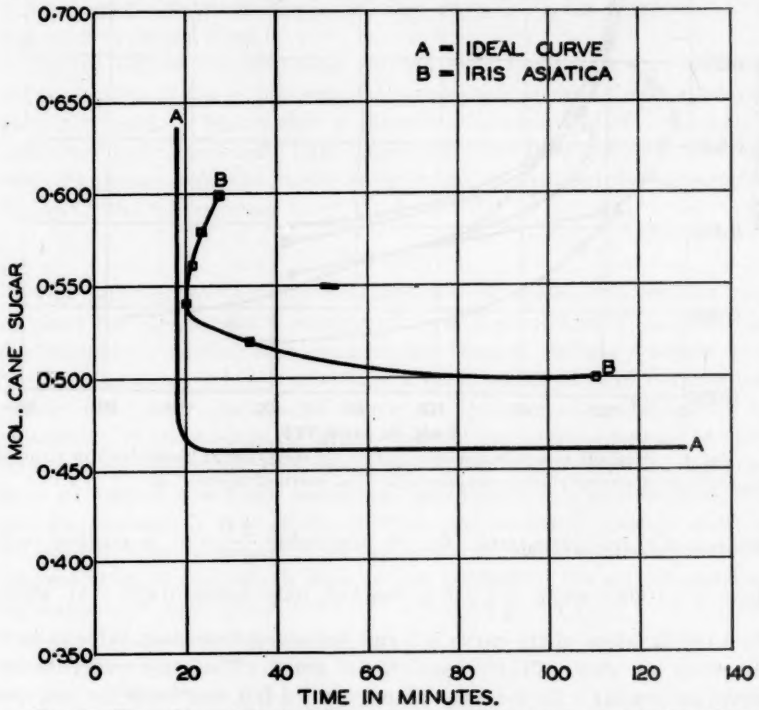


FIG. 1. Time of immersion in different concentrations of cane sugar before observed inception of plasmolysis: *A*, ideal curve for determination of osmotic pressure by the plasmolytic method; *B*, observed curve for stripped preparation of *Iris asiatica*.

curve for *Iris asiatica* (fig. 1, *B*). Here the critical concentration lies somewhere between 0.500 and 0.530 M, since at 0.500 it required two hours for plasmolysis to appear while at 0.530 M and all higher concentrations a similar state was reached within 20–30 minutes. The slope of the curve above $C = 0.530$ was thus ∞, while below that value it was 0 (approximate). If such were always the case, plasmolysis would be a satisfactory means of measuring osmotic value. This, however, is not the case. In the curve

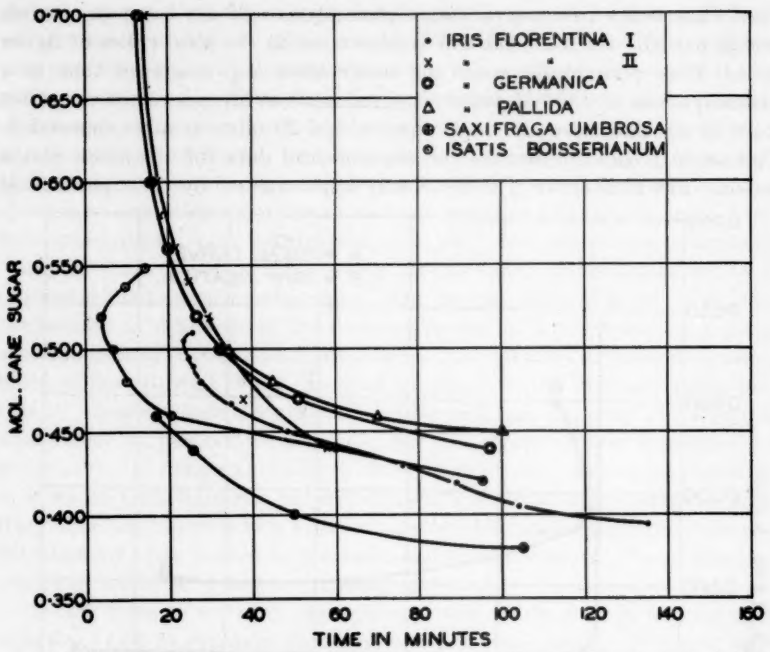


FIG. 2. Observed concentration-time curves for inception of plasmolysis in stripped preparations of several plants; compare with ideal curve of figure 1, *A*.

obtained for *Iris germanica* (fig. 2), the value $\frac{\Delta C}{\Delta T} = \infty$ is reached only above $C = 0.600$, while $\frac{\Delta C}{\Delta T} = 0$ is reached only below 0.425. At about $C = 0.480$ the slope of the curve is 1 and deviations from that value in both directions are slow. There is no *critical* point. The other experimental curves are similar. Of the seven plants studied *Iris asiatica* is the only one which gave data showing any semblance of a critical point. Since the whole method rests on the assumption of the existence of such a point, its invalidity is obvious.

The plasmolytic method as formerly used is therefore invalid because it does not deal with tissues in normal, healthy condition, because it frequently does not differentiate between true osmotic phenomena and pseudoplasmolytic mechanical phenomena, and because when these factors are eliminated and the phenomenon considered as a concentration-time relationship it gives critical values in only rare instances. It seems very doubtful whether it will ever be possible to eliminate all of these objections satisfactorily.

A curious phenomenon, a slower appearance of plasmolysis in high concentrations than in low ones, is illustrated in figure 1 (*Isatis boisserianum* and *Iris florentina*). This anomaly was observed in a number of experiments, and in every case the cells appeared healthy, and except for the time relation plasmolysis appeared to follow the normal course. The explanation is not clear, but temperature seems to play some part in the process, as the delay of plasmolysis in high concentrations did not occur in tests made somewhat late in the day when the room temperature was higher, or on warm days.

In yet higher concentrations the cells sometimes show internal plasmolysis and sometimes fail entirely to plasmolyze, while still appearing perfectly healthy. This failure to plasmolyze is not a case of deplasmolysis which has been overlooked. Cane sugar does enter these cells but only after some 36 hours, when its entry is probably conditioned by pathological change in the cell membrane.

Summary

The relationship between concentration of plasmolyticum and time required for plasmolysis is considered from a theoretical standpoint and the conclusion reached that considerable time is necessary before equilibrium is attained between a cell and a surrounding fluid of only slightly greater or slightly less osmotic strength. Data on this relationship are presented. When mechanical injury and the pathological actions of fluids from ruptured cells are eliminated, and when temperature and light are those of normal laboratory conditions, plasmolysis is a slow process; and limiting plasmolysis is so much deferred that secondary changes must be expected to have occurred in the cells such as would render invalid the interpretation of plasmolysis data as any measure of the osmotic value of the contents of plant cells.

FOREST PRODUCTS RESEARCH LABORATORY
PRINCES RISBOROUGH, BUCKS
ENGLAND

LITERATURE CITED

1. BECK, W. A. Osmotic pressure, osmotic value and suction tension. Plant Physiol. **3**: 413–440. 1928.
2. ————————. Cane sugar and potassium nitrate as plasmolysing agents. Protoplasma **1**: 15–72. 1926.
3. ERNEST, ELIZABETH C. M. Suction-pressure gradients and the measurement of suction pressure. Ann. Bot. **45**: 717–731. 1931.
4. FITTING, HANS. Die Wasserversorgung und die osmotischen Druckverhältnisse der Wüstenpflanzen. Zeitschr. Bot. **3**: 209–275. 1911.

5. HANNIG, E. Untersuchungen über die Verteilung des osmotischen Drucks in der Pflanze in Hinsicht auf die Wasserleitung. Ber. deut. bot. Ges. **30**: 194–204. 1912.
6. HÖFLER, KARL. Eine plasmolytisch-volumetrische Methode zur Bestimmung des osmotischen Wertes von Pflanzenzellen. Denkschr. Kais. Akad. Wiss. Wien, math.-nat. Kl. **95**: 99. 1918.
7. ————. Über Eintritts- und Rückgangsgeschwindigkeit der Plasmolyse, und eine Methode zur Bestimmung der Wasserpermeabilität des Protoplasten. Jahrb. wiss. Bot. **73**: 300–350. 1930.
8. ILJIN, W. S. Die Regulierung der Spaltöffnungen in Zusammenhang mit der Veränderung des osmotischen Druckes. Beih. bot. Centr. **32**: 15–35. 1915.
9. URSPRUNG, A., and BLUM, G. Über die Verteilung des osmotischen Wertes in der Pflanze. Ber. deut. bot. Ges. **34**: 88–104. 1916.
10. DE VRIES, HUGO. Eine Methode zur Analyze der Turgorkraft. Jahr. wiss. Bot. **14**: 427–601. 1884.
11. ————. Über eine neue Anwendung der plasmolytischen Methode. Bot. Zeitg. **46**: 393–397. 1888.
12. WEBER, F. Plasmolyse-Zeit-Methode. Protoplasma **5**: 622–624. 1929.
13. ————. Plasmolysezeit und Lichtwirkung. Protoplasma **7**: 256–258. 1929.

PANTOTHENIC ACID AS A NUTRILITE FOR GREEN PLANTS

ROGER J. WILLIAMS AND EWALD ROHRMAN

Introduction

Ever since the work of BOTTOMLEY on the stimulating effect of extracts of bacterized peat on the growth of green plants, there has been some interest in the possibility that green plants may require an outside source of specific organic material. The preponderance of opinion (**1, 2, 6**) seems to favor the idea that, while stimulating effects may exist, the green plant is able to grow without any such outside stimulus and hence is independent in accordance with the classical view.

It has been suggested (**6**) that the term "auximone," coined by BOTTOMLEY, should be dropped from the literature because the substances are not indispensable and the experimental data from which their existence was inferred were based upon an unfavorable salt balance. It is claimed that auximones, as conceived by BOTTOMLEY, do not really exist. In this connection we again call attention to the advantage in the term *nutrilite* (**3**), which carries no presuppositions except that the vitamin-like substance functions in nutrition.

Up to the present, so far as we are aware, no substantial clue which will stand scrutiny has been obtained as to the chemical nature of the substances which are presumed to exist and to stimulate the growth of green plants.

Experimental work

The first suggestion that pantothenic acid (**4**) might be involved in this problem came from a study of the pantothenic acid content of dormant and sprouting potatoes. It was found that while the pantothenic acid is rather uniformly distributed throughout the tissue of dormant potatoes, during sprouting there is a distinct mobilization of the acid toward the sprouting end. This suggested that it might have a growth stimulating function as is the case with yeast.

Preliminary work, conducted about the same time by Dr. W. L. POWERS of the Department of Soils, showed that one of our relatively crude concentrates had a stimulating effect on alfalfa seedlings. The details of these and other tests will be published elsewhere.

Through the initiative of the junior writer, experiments were conducted with an aquatic plant which, so far as we know, has not been used for physiological study. Young plants of *Ricciocarpus natans* were collected from an aquarium and later from a marshy lake, and cultured on

Detmer's solution. The first effects were observed visually. When the young plants grow the two lobes separate as a new lobe grows between them. It was noted that this evidence of growth was much more apparent in cultures to which from 10 to 10,000 parts per billion of a crude pantothenic acid concentrate (calcium salt potency of 70) was added. The best result was obtained with 1 part per million and the difference became definitely noticeable on the third day. In another experiment there was visual evidence (recorded after 12 days) of improved growth when as little as 1 part per billion of a refined preparation (potency 5000) was added. When 10 and 100 parts per billion were added the growths were better than the control but not so good as when only 1 part per billion was present.

In a third experiment, two carefully selected plants of uniform size were grown in each of four dishes. Those to which additions of the refined preparation just described were made showed some increased growth in 5 days; and after 9 days the plants were blotted uniformly between filter papers and weighed in a closed vessel on a micro balance, with the results indicated in table I.

TABLE I

EFFECT OF PANTOTHENIC ACID ON GROWTH OF *RICCOCARPUS NATANS*

	WEIGHT OF PLANTS
Blank	0.002545
1 p. p. billion	0.003210
10 p. p. billion	0.004350
100 p. p. billion	0.003840

It is evident that 100 parts per billion is an overdose for this particular preparation. It should be noted also that while the medium used may not be ideal for the liverwort, the amount of material added is extremely minute, and its effect must therefore be specific.

Further experiments indicated that the liverwort used in these experiments is not a very favorable plant for continuous laboratory culture, because the young plants are available only after sporulation and this event cannot be controlled in the laboratory. The physiological condition of the plants is an important factor. The plants which showed the most striking responses were those obtained from the aquarium. Those taken from the natural habitat were less responsive, and after keeping for a few weeks the plants in one case were apparently unaffected by the more refined preparation, except that 100 parts per billion actually inhibited growth.

Within a few days after these experiments had been completed, a communication was received from Professor W. J. ROBBINS indicating that in preliminary experiments he had obtained positive growth stimulating results with a pantothenic acid preparation which we had furnished. His study utilizing isolated root tips from corn will doubtless be published shortly.

Discussion

These studies with four different plants (including the potato), even though they are not exhaustive, would seem to indicate that pantothenic acid plays a rôle in the growth of green plants generally. This idea is strengthened by the evidence previously presented (**4**), that pantothenic acid is a constituent of all types of living matter.

To the plant physiologist mainly will fall the problem, which will probably prove an intricate one, of finding precisely how pantothenic acid functions. The writers cannot answer this question and available facilities will probably severely limit our further work along this line. We wish, however, to mention a few observations which are an outgrowth of our limited experience with green plants and our more extended acquanitance with the chemical factors influencing yeast growth.

ORIGIN OF PANTOTHENIC ACID.—The observation has been recorded elsewhere (**4**) that *Aspergillus niger* produces pantothenic acid as it grows upon a synthetic medium. We have also observed that several bacteria and other molds which can grow upon synthetic media also produce it. It is not surprising, therefore, that the substance is present in soils and especially abundant in those soils which are rich in organic matter. Our experience would lead us to suspect that it is produced neither by higher plants nor by animals. We have conducted careful experiments to prove whether or not it is produced as yeast grows (extremely slowly) in a synthetic medium or one with known low pantothenic acid content. The results indicate that it is not produced. Yeast grown on a medium low in pantothenic acid content contains very little pantothenic acid, as low as one-fiftieth as much as "normal" yeast and even less than that introduced originally into the medium. The medium at the end of the growth period was practically devoid of pantothenic acid. It seems that under these conditions the pantothenic acid present in the mother cells is distributed to the daughter cells, and that growth reaches practically a standstill in the course of a few days, owing to its very low concentration.

IS PANTOTHENIC ACID INDISPENSABLE OR MERELY STIMULATIVE?—In the light of the experiments just cited, one can draw his own conclusions as to the indispensability of pantothenic acid for yeast growth. If the conclusion is that the effect is that of a stimulant, the interest in the stimulating

substance should not be diminished, because no one can question but that under anything approaching natural conditions yeast is always stimulated by pantothenic acid, whether or not it is capable of growing very slowly without that stimulation.

To us the possibility that pantothenic acid is stimulative rather than indispensable to green plants should not lessen interest in it. Under natural conditions plants are doubtless stimulated by the substance because it is found in soils generally and the organisms of the soil are capable of producing more when the available supply runs low, of course, provided that the soil is suitable for the growth of these organisms. Just how long a plant can grow and eke out an existence without a further supply of organic matter beyond that supplied by the seed is not very important, if under ordinary circumstances it obtains specific organic matter from outside sources and grows normally because of this exogenous supply.

ARE OTHER NUTRILITES INVOLVED IN GREEN PLANT STIMULATION BESIDES PANTOTHENIC ACID?—Our experimental evidence with regard to this point is scanty, yet we have observed in work with the liverworts that an extract of potato seemed to have a stimulating effect well beyond that which could be accounted for by its pantothenic acid content alone. Since the physiological condition of the test organism could not be standardized, the results were not of a quantitative nature, and a definite conclusion would therefore be dangerous.

From our work with yeasts we should not be surprised if factors other than pantothenic acid were involved. Not all of the various strains of *Saccharomyces cerevisiae* are stimulated markedly by pantothenic acid alone. Other complementary factors may be necessary to produce even approximately normal growth. In the case of two yeasts, crystalline vitamin B_1 fills this need. What the nature of the complementary substance is in the case of green plants we do not know, but we expect to be able to answer the question as to their existence and nature by further experimentation. From analogy with yeast growth experience, we should expect that plant growth stimulation could be induced much more markedly than has been possible if all the unknown factors were supplied to the growing plant.

EFFECT OF TOXIC FACTORS.—Not only with yeast but in our limited experiments with green plants we have observed the influence of inhibiting substances. In yeast growth experiments we are constantly confronted with such effects. We have natural extracts which are devoid of any ability to stimulate yeast growth because they contain toxic material which is specific for a given yeast. By fractional electrolysis (5) we are able to prepare very active stimulating material from the same extract. Our experiments indicate that such results will carry over to experiments with green plants and will significantly modify results. There is also the possibility

that an unfavorable balance between various complementary growth-stimulating substances may result in growth inhibition. This appears to take place with yeast in some cases.

OREGON STATE AGRICULTURAL COLLEGE
CORVALLIS, OREGON

Summary

1. Pantothenic acid has been found in preliminary experiments to have a growth stimulating effect on green plants.

2. The questions of whether it is stimulative or indispensable, where it originates in nature, and the probability of the existence of other plant nutrilites, are discussed.

LITERATURE CITED

1. CLARK, N. A. "Auximones" and the stimulation of *Lemna* by organic matter. Science n. s. **71**: 268–269. 1930.
2. HARDY, F. Auximone nutrition (review). Trop. Agr. (Trinidad) **10**: 18–21. 1933.
3. WILLIAMS, R. J. "Nutrilites." Science n. s. **67**: 607–608. 1928.
4. ————, LYMAN, C. M., GOODYEAR, G. H., TRUESDAIL, J. H., AND HOLADAY, D. "Pantothenic Acid," A growth determination of universal biological occurrence. Jour. Amer. Chem. Soc. **55**: 2912–2927. 1933.
5. ————, AND TRUESDAIL, J. H. The use of fractional electrolysis in the fractionation of the "bios" of WILDIERS. Jour. Amer. Chem. Soc. **53**: 4171–4181. 1931.
6. WOLFE, H. S. The auximone question. Bot. Gaz. **81**: 228–231. 1926.

SOLUBILITY OF POTASSIUM IN CORN TISSUES[1]

V. H. Morris and J. D. Sayre

Introduction

In a recent paper (**6**) it was suggested that the composition of succulent plant tissues with respect to any constituent in true solution in the cell sap can be calculated from an analysis of sap expressed from the tissues. In applying this method of analysis to studies of the mineral nutrition of the corn plant, it was necessary to ascertain whether the potassium was entirely in solution in the cell sap. If this situation obtained, the method of analysis could be used to determine the total potassium content,—in some investigations a much simpler and more convenient procedure than the usual ash analysis involving drying and grinding the sample.

In investigating the potassium content of expressed corn sap, data were obtained which are believed to furnish an answer to the question as to whether potassium occurs in corn tissues entirely in expressible form or whether it may be partially fixed by association with cell constituents such as lignin. The data are presented in this paper.

The literature bearing on this subject is generally interpreted as leading to the conclusion that potassium occurs only in solution in the cell sap, and probably in inorganic form (**5, 1**). Recently, however, McGeorge (**4**) presented evidence suggesting the possibility of a system of base exchange in living plant tissues similar to that already demonstrated for soils, where a correlation has been found between such factors as lignin content of the organic matter and exchange capacity. Inozemtzev (**3**) has concluded from electrodialysis studies that the greater part of the potassium of plants exists in a complex nondialyzable form, and that the dialyzable potassium is probably in an organic form.

Material and methods

The material consisted of samples taken during a seasonal study of the distribution of potassium in the different parts of the corn plant. A single cross adapted to Wooster conditions was grown on a fairly uniform soil in a good state of fertility. On each sampling date several plants were brought into the laboratory and separated into the following parts: blade, sheath, upper stem (above ear), lower stem, tassel, husks, grain, and cob. Each part was finely cut in an electric foodchopper. The potassium content was calculated from an analysis of the sap expressed from portions of the ground tissue, as previously described (**6**). Samples of the ground tissues also were

[1] Investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry and Department of Agronomy, Ohio Agricultural Experiment Station.

dried in a steam chamber and then prepared for analysis. Portions of a few of the samples also were extracted with hot water or with 5 per cent. alcohol, using 50 gm. of the green tissue in Soxhlet extractors and making the extract to a final volume of 500 ml.

Potassium was determined in the various kinds of samples after ignition with sulphuric acid, using 10-ml. aliquots of the expressed sap, 2.5 gm. of dried tissue, and 100 ml. of the extracts. The ashed samples were taken up in hot dilute HCl, filtered, and washed into 250-ml. volumetric flasks. The potassium was precipitated from 50-ml. aliquots by the chloroplatinate method (**2**), first removing the calcium as the oxalate. All samples were taken in duplicate and each value reported is the mean of the duplicates.

Results

It was desirable to determine first whether there was any potassium which was in an insoluble form or held by adsorption and that could be obtained by continued extraction but not by the sap expression method. The results obtained with a series of samples, reported in table I, do not indicate the

TABLE I

POTASSIUM CONTENT OF CORN TISSUE AS DETERMINED BY SAP EXPRESSION, EXTRACTION, AND ASH ANALYSIS OF DRIED TISSUE (PERCENTAGES ON GREEN WEIGHT BASIS)

DATE	TISSUE	SAP EXPRESSION	EXTRACTION		TOTAL IN DRIED TISSUE
			H_2O	5% ALCOHOL	
		%	%	%	%
June 30	Whole plant	0.23	0.25	0.25	0.27
July 7	Blade	0.35	0.36	0.30	0.34
July 14	Blade	0.43	0.45	0.35	0.46
	Stem	0.18	0.19	0.19	0.18
July 21	Blade	0.36	0.38	.....	0.40
	Sheath	0.26	0.26	.....	0.25
	Upper stem	0.18	0.13	.....	0.18
	Lower stem	0.13	0.11	.....	0.15
July 28	Blade	0.43	0.45	.....	0.49
	Lower stem	0.17	0.13	.....	0.17
	Ear	0.20	0.15	.....	0.20

presence of potassium which could be extracted but not expressed. Furthermore, as shown in the same table, comparison with the analysis of dried tissue shows that all of the potassium was obtained by both extraction and sap expression.

Further evidence that all the potassium is in solution in the cell sap and consequently can be determined in corn tissue by the sap expression method,

as well as by analysis of dried tissue, is afforded by the data from a series of 56 samples, the results of which are summarized in table II. Although the

TABLE II

POTASSIUM CONTENT OF CORN TISSUES AS DETERMINED BY SAP EXPRESSION AND ASH ANALYSIS OF DRIED TISSUE (PERCENTAGES ON GREEN WEIGHT BASIS)

TISSUE	NUMBER OF SAMPLES AVERAGED	SAP EXPRESSION	TOTAL IN DRIED TISSUE
		%	%
Blade	10	0.389	0.414
Sheath	7	0.319	0.317
Upper stem	7	0.176	0.189
Lower stem	9	0.184	0.178
Husks and silks	4	0.130	0.157
Grain	3	0.190	0.180
Cob	5	0.152	0.192
Hybrids (stem tissue)	11	0.165	0.185
Mean of all samples		0.226	0.240

mean of the 56 samples is slightly higher for total potassium in dried tissue, the difference (0.014) is not significant, since the standard deviation of the difference between the two methods is 0.029.

The only samples with differences large enough to approach significance were the last two samples of cobs, taken on August 18 and 25. The potassium contents by the sap expression method were 0.128 and 0.143 respectively, as compared with 0.204 and 0.247 for dried tissues. Whether these differences are due to discrepancies in technique, to mechanical difficulties in handling this kind of tissue, or actually represent the presence of potassium held in such a way that it could not be expressed, cannot be determined from these data. That it may well be due to the mechanical difficulty of expressing sap from cobs rather than to the high percentage of lignin in these tissues is indicated by the fact that the first three samples of cobs showed good agreement between the methods.

With the exception noted, the data indicate that the potassium in corn tissues is in solution in the cell sap and thus can be determined by analysis of expressed sap as well as by analysis of dried tissue. Since all the samples were first ignited with sulphuric acid, however, it is possible that some of the potassium might be in solution in organic form, or at least unionizable. Data obtained by the direct precipitation of the potassium in a cleared portion of the sap by sodium cobaltinitrite are presented in table III. The results by the different methods are in good agreement. From these it appears that all the potassium in corn tissues is in such a form that it can be directly precipitated by regents such as sodium cobaltinitrite, indicating that it is in true solution and ionizable.

TABLE III

POTASSIUM CONTENT OF CORN TISSUE DETERMINED BY DIRECT PRECIPITATION FROM EXPRESSED SAP AS COBALTINITRITE COMPARED WITH ASHED EXPRESSED SAP AND DRIED TISSUE ANALYSIS

Tissue	Total in dried tissue	Sap expression	
		Gravimetric	Colorimetric
	%	%	%
Blade	0.40	0.36	0.36
Sheath	0.25	0.25	0.26
Upper stem	0.18	0.18	0.19
Lower stem	0.12	0.13	0.14
Tassel	0.31	0.27	0.28

The assumption that a base exchange system exists in the plant implies, by analogy with the well known soil system, a tying up of bases, potassium for example, in insoluble form in absorption complex or chemical combination. From the data presented in this paper it may be concluded that with respect to potassium such a system does not obtain in the corn plant.

Summary

1. The potassium in corn tissue is entirely in solution in the cell sap and consequently can be determined by analysis of expressed sap as well as by extraction or dried tissue analysis.

2. With the possible exception of cob tissue, there is no evidence of the presence in corn tissues of any insoluble, fixed, or unionizable forms of potassium.

OHIO AGRICULTURAL EXPERIMENT STATION
WOOSTER, OHIO

LITERATURE CITED

1. Annual review of biochemistry. Vol. I. (p. 628) 1932.
2. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMSITS. Official and tentative methods of analysis. Published by the Association. Washington, D. C. 2d ed. 1924.
3. INOZEMTZEV, S. L. Nature of potassium compounds in plants. Ergeb. Veg. Lab. arb. Pryanishnikov **15**: 85–101. 1930. Chem. Abstr. **27**: 5368. 1933.
4. MCGEORGE, W. T. Chemical equivalent base exchange reactions in plants. Plant Physiol. **7**: 119–124. 1932.
5. MILLER, E. C. Plant physiology. p. 254. McGraw-Hill, New York. 1932.
6. SAYRE, J. D., and MORRIS, V. H. Use of expressed sap in determining the composition of corn tissue. Plant Physiol. **7**: 261–272. 1932.

PHOTOELECTRIC APPARATUS FOR MEASURING LEAF AREAS[1]

DONALD E. H. FREAR

(WITH THREE FIGURES)

The need to measure accurately the area of several thousand apple leaves in connection with a study of the deposit of spray residues led to a search of existing methods of leaf area measurement. The several methods described in the literature failed to satisfy the requirements, inasmuch as accuracy in area measurements was apparently to be secured only by methods too lengthy to be of use in this study.

Existing methods for measuring leaf areas include the following: (1) Measurement of the leaves along their axes, the area obtained by multiplying these figures being corrected by a factor; (2) planimeter measurements; (3) tracing or otherwise transferring the leaf outline to paper of known weight per unit of area and later determining the area of the tracings by their weight; (4) leaf-punch methods, in which definite areas of the leaf surface are removed by a punch or die.

The first of these methods is not sufficiently accurate, since variations in leaf shape among individual leaves of the same species is sufficient to introduce gross errors into the calculation. The second and third methods are extremely time-consuming, depend largely for their accuracy on the skill and dexterity of the person using them, and are adapted only for measuring the areas of small numbers of leaves. Circles or other geometric figures cut from the leaf surface with a punch or die are subject to errors arising from the obvious fact that the leaf presents neither a uniform surface nor an interior structure free from gross tissue differentiation.

The use of the photoelectric cell in a variety of ways in recent years led the writer to consider the possibility of such a device for measuring leaf areas, inasmuch as the area would be a direct function of the amount of light intercepted if the leaf were placed in the path of a beam of light. While a device was being perfected, a short news article appeared describing a similar apparatus, built by WITHROW, and it was learned that a paper describing a similar apparatus had been presented at the Boston meeting of the American Society of Plant Physiologists. Definite information concerning the construction of this apparatus could not be secured, however, and the present apparatus has been developed independently, and has been used satisfactorily for the past season.

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, as Technical Paper no. 677.

Apparatus

A rectangular box 72 cm. high, 22 cm. deep, and 22 cm. wide was constructed from plyboard, with a tight fitting door at the front. The interior of the box was painted white and near the top a number of holes were bored for ventilation. A perpendicular section through the apparatus is shown in figure 1. A movable shelf *A*, to which were attached five 60-watt 110-

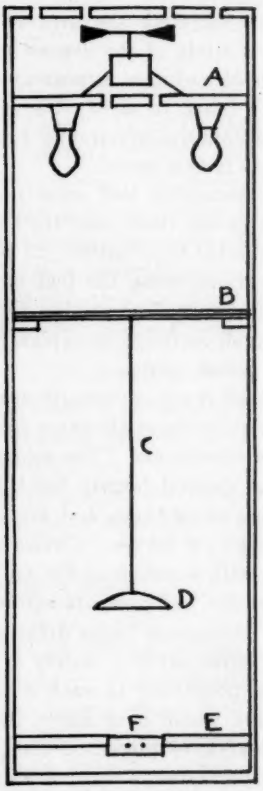


FIG. 1. Section through apparatus.

volt inside frosted electric lamps, was inserted near the top of the box. These lamps may be connected to a constant wattage transformer so that current variations may be reduced to a minimum. On this shelf was placed a small electric fan to circulate the air. Across the middle of the box was placed a pane of frosted glass, of such size that it exactly fitted the interior of the box, but masked so that only a circular opening approxi-

mately 18 cm. in diameter was available to transmit light (fig. 1 *B*). A small hole was drilled in the exact center of the circle of frosted glass so circumscribed, and a hemispherical baffle *D* was suspended by a glass rod *C*, approximately 15 cm. below the frosted glass plate. A sheet of heavy plate glass of the same size as the frosted glass was laid on top of the latter.

At the bottom of the box, about 4 cm. above the true bottom, a false bottom *E* was built, in the center of which a hole large enough to accommodate the photoelectric cell was cut. This photoelectric cell, a Weston Model 594, was connected with a microammeter having a capacity of 200 microamperes.

The purpose of the hemispherical baffle is to deflect all light rays passing through the frosted glass which would otherwise fall directly upon the surface of the photronic cell. This makes it possible for the cell to measure the *diffused* light in the lower chamber, rather than to give erratic readings because of differences in the location of shaded areas. Such differences would exist if the object causing the shading (in this case the leaves) were located in different places on the ground glass.

The intensity of the illumination in the lower chamber may be regulated by raising or lowering the bank of lamps at the top of the box.

Standardization

In standardizing the instrument, the light bulbs are connected to a 110-volt current source and adjusted so that the reading of the microammeter connected with the photronic cell is nearly the maximum of the instrument. This original reading is recorded. A section of definite area cut from a leaf similar to those to be measured is then placed dorsal side uppermost, upon the ground glass plate, and held in a flat position by the sheet of plate glass. The reading of the microammeter is again taken and the process repeated, adding a section of leaf of known area each time until the ground glass plate is covered to its capacity. A curve is then prepared, plotting the percentage of light cut off against leaf area. The percentage of light cut off is calculated by subtracting the microammeter reading taken for any given leaf area from the original reading, and dividing this by the original reading.

Variations in the electric current cause fluctuations in the reading of the microammeter, but it has been found that over the relatively short time necessary to make the standardization or to take the readings on a series of whole leaves, as will be described later, these fluctuations are not large enough to cause serious error. The use of the constant wattage transformer mentioned reduces these fluctuations greatly. It is wise to repeat the standardization several times, however, so that each point on the curve rep-

resents the mean of several readings on the microammeter. A typical standardization curve is shown in figure 2.

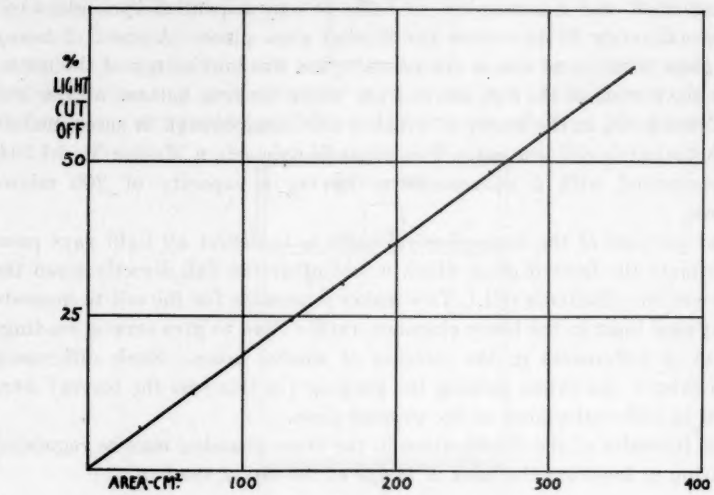


FIG. 2. Standardization curve.

Measurement of leaf areas

Having constructed a curve for the type of leaves to be measured, the actual measurement of the leaf area is carried out in a manner similar to the standardization. The original reading of the microammeter is taken without any material on the ground glass plate to interfere with the passage of the light. The leaves to be measured are then placed on the ground glass, one at a time, or, if the total area of a number of leaves is desired, several leaves at a time, and the second reading of the instrument taken. It has been found that with an apparatus of the dimensions given, four or five mature apple leaves may be placed upon the ground glass at one time, and the area of 100 leaves measured in less than 15 minutes. To guard against wide fluctuations in current, it is wise to take original readings after each five sets of leaf area readings.

The percentage of light cut off by each leaf or group of leaves is then calculated, and by reference to the standard curve, the area is determined. An example of the calculation is as follows:

Original reading ..... 188 microamperes
Reading with 4 leaves ..... 103 microamperes

$$\frac{(188-103)}{188} = 44.0\% \text{ of the original light cut off.}$$

Referring to the curve given in figure 2, this indicates an area of 238 square centimeters.

Discussion

In building this apparatus an attempt has been made to avoid any unsymmetrical construction which might cause shadows, or tend to give a different light intensity on the photronic cell owing to a variation of the position of the leaf or leaves to be measured when placed upon the ground glass. With the instrument here described, it makes no appreciable difference whether the leaf is in the center of the circular opening on the ground glass or far to one side.

The method of standardization of the instrument may introduce serious errors in the determination of leaf areas by any photoelectric method. Leaf tissue, except perhaps in rare cases, is appreciably translucent. In addition, the reflection of light from the surface of a leaf is characteristically different from the reflection from many other materials. In fact, the reflection of light from the upper surface of a leaf is usually different from the reflection from the lower surface. This is particularly true if the lower surface is pubescent. For these reasons, then, the standardization of the instrument against known areas of opaque material, or against material of different color or reflectivity from that of the leaf surface, may lead to inaccurate results. Figure 3 shows typical curves obtained by standardizing the instrument with known areas of black paper (*A*); white cardboard

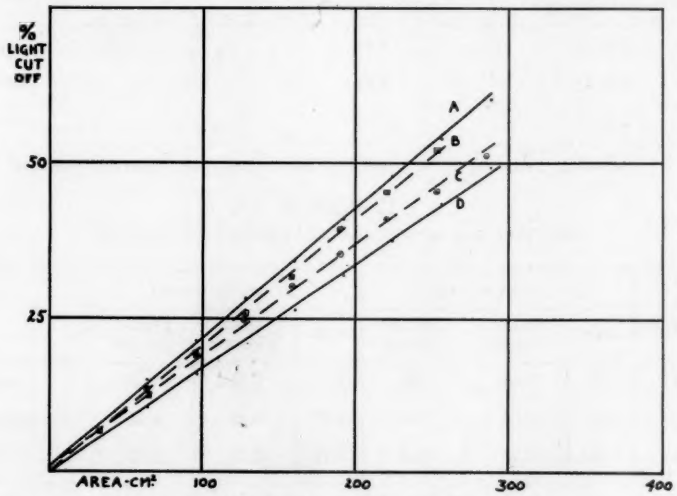


FIG. 3. Comparative standardization curves.

(*D*); and two types of leaf tissue, one thick and practically opaque (*B*) and the other relatively thin and translucent (*C*).

The use of alternating current to provide illumination admittedly increases the error of measurement. It is possible, however, through the use of a transformer to keep this at a minimum, and frequent checking of the current through microammeter readings helps reduce this error.

This apparatus has been used satisfactorily to measure the area of more than 10,000 apple leaves during the past season, with an error of approximately 3 per cent., as determined by replicate area measurements on the same leaves.

Summary

An apparatus is described for the measurement of leaf areas by means of the photoelectric cell. The chief advantages of the method are its rapidity and simplicity, with a high degree of accuracy. The methods of standardization and calculation are given in detail. The apparatus has been used to determine the area of more than 10,000 apple leaves, with an error of approximately 3 per cent.

Department of Agricultural and Biological Chemistry
Pennsylvania State College

BRIEF PAPERS

NOTES ON THE SUCROSE CONTENT AND DEXTROSE–LEVULOSE RATIO OF CALIFORNIA DRIED PRUNES[1]

The quality of dried prunes in California is to some extent related to the districts in which the fruit has grown. The principal variety grown in all sections is the French prune d'Agen. The four principal prune-growing districts are the Santa Clara and the Napa-Sonoma which are often designated as "inside" districts, and the Sacramento and the San Joaquin valleys which are often called "outside" districts in the prune trade. The inside districts are near the coast where the temperatures are lower. Less irrigation is necessary or customary in the coastal or inside districts, it is more common to use short pruning methods, and the crops average smaller. In the inside districts the fruit normally falls to the ground when in best condition for drying, while for some undetermined reason the fruit in the outside districts in large measure remains on the tree until deterioration is marked unless shaken or knocked to the ground.

TABLE I

TOTAL SUGAR CONTENT* OF SAMPLES OF THE 1932 CROP

	INSIDE DISTRICTS	OUTSIDE DISTRICTS
Average	49.4	40.5
Maximum	53.3	45.1
Minimum	46.4	37.3

* Expressed as invert sugar, adjusted to a basis of 20 per cent. moisture.

TABLE II

TOTAL SUGAR CONTENT* OF SAMPLES OF THE 1933 CROP

	INSIDE DISTRICTS			OUTSIDE DISTRICTS			ALL DISTRICTS
	SANTA CLARA	NAPA-SONOMA	ALL	SACRAMENTO	SAN JOAQUIN	ALL	
Average	54.7	49.5	52.1	45.1	45.8	45.5	49.4
Maximum	59.4	51.5	59.4	47.2	50.9	50.9	59.4
Minimum	47.7	47.2	47.2	42.7	42.3	42.3	42.3

* Expressed as invert sugar, adjusted to a basis of 20 per cent. moisture.

[1] This brief paper was prepared by Professor NICHOLS shortly before his untimely death in November, 1934.

TABLE III

SUCROSE CONTENT OF SAMPLES OF THE 1933 CROP

INSIDE DISTRICTS				OUTSIDE DISTRICTS			
SANTA CLARA		NAPA-SONOMA		SACRAMENTO		SAN JOAQUIN	
COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE	COUNT PER LB.	SUCROSE
	%		%		%		%
74	0.95	37	10.26	39	3.23	64	0.95
		46	6.18	49	7.79	86	6.84
44	4.37*	61	10.35	66	8.08	120	4.18
49	0.88*	82	8.17	90	7.69		
73	5.42*	107	8.64	109	4.18	60	3.61
77	6.18*					83	5.13
122	12.05*	40	1.52	39	3.99	98	9.31
		64	7.50	70	7.79		
40	1.33	45	6.08	47	6.19	47	1.52
49	7.12	80	3.23	88	4.84	68	3.61
68	7.60	108	2.95	108	10.74	78	1.71
78	4.94					113	2.18
104	3.61	79	4.27				
		68	7.22				
116	1.90	102	5.50				
		133	2.37				
68	5.22						
93	1.16						
113	5.13						
Av.	4.51	Av.	6.02	Av.	6.42	Av.	3.90
Max.	12.05	Max.	10.35	Max.	10.74	Max.	9.31
Min.	0.88	Min.	1.52	Min.	1.90	Min.	0.95
Inside districts:		Average	5.24	Maximum	12.05	Minimum	0.88
Outside districts:		Average	5.06	Maximum	10.74	Minimum	0.95
All districts:		Average	5.12	Maximum	12.05	Minimum	0.88

* Samples in subgroups within columns are of different sizes from single grower's delivery.

The inside districts have the reputation of producing fruit of higher quality than that of the outside districts. It has been shown by HILTNER and HATHERELL (**2**) and by GALE and CRUESS (**1**) that the total sugar content of dried fruit from the inside districts is higher. This has been confirmed by NICHOLS and REED (**5**), who also reported that the texture of the flesh is more solid and the specific gravity of the whole fruit higher in the fruit from inside districts. These facts apparently account in part for the trade preference for such fruit.

It was thought that not only the total sugar content but also the sucrose content and the dextrose-levulose ratio, on which some data have been published by MRAK, SMITH, and HENRIQUES (**4**), might be related to the district

TABLE IV

DEXTROSE-LEVULOSE RATIO IN SAMPLES OF THE 1933 CROP

INSIDE DISTRICTS				OUTSIDE DISTRICTS			
SANTA CLARA		NAPA-SONOMA		SACRAMENTO		SAN JOAQUIN	
COUNT PER LB.	D/L RATIO	COUNT PER LB.	D/L RATIO	COUNT PER LB.	D/L RATIO	COUNT PER LB.	D/L RATIO
128	2.15	37	7.25	39	2.82	64	1.98
44	2.29*	46	4.10	46	2.86	86	2.25
49	2.17*	61	4.29	66	3.35	120	2.54
73	1.87*	82	4.08	90	3.07	60	2.58
77	1.76*	107	5.43	109	2.22	83	2.75
122	2.01*	40	2.57	39	2.67	98	3.60
40	1.64	45	4.28	47	3.17	47	2.77
49	2.28	64	3.86	70	2.46	68	3.15
68	2.53	80	2.52	88	2.42	78	3.22
78	1.98	108	3.09	108	2.85	113	3.59
104	2.02	79	2.65				
116	2.35	68	2.42				
68	1.68	102	2.69				
93	2.32	133	2.42				
113	1.63						
Av.	2.04	Av.	3.69	Av.	2.79	Av.	2.85
Max.	2.53	Max.	7.25	Max.	3.35	Max.	3.60
Min.	1.63	Min.	2.42	Min.	2.22	Min.	1.98
Inside districts:		Average	2.60	Maximum	7.25	Minimum	1.63
Outside districts:		Average	2.85	Maximum	3.60	Minimum	1.98
All districts:		Average	2.63	Maximum	7.25	Minimum	1.63

* Samples in subgroups within columns are of different sizes from single grower's delivery.

of origin. Determinations of sucrose and dextrose-levulose ratio, as well as specific gravity and total sugar, were therefore made on about 60 samples representing fairly well the 1933 and 1934 crops of the principal four California districts.

The total sugar content of the 1932-crop samples was determined by the SHAFFER-HARTMANN method (**6**); those of the 1933-crop samples by the volumetric permanganate method of the Association of Official Agricultural Chemists. In both cases the total sugar was expressed as invert sugar. The 1933-crop samples were examined for total reducing sugars before inversion, also for dextrose by the LOTHROP and HOLMES (**3**) method.

The possible relation between specific gravity and total sugar content was studied, and while the correlation is by no means close there is a definite

tendency for high sugar content and high specific gravity to be associated, especially in prunes from the outside districts.

The variations in total sugar content of 1932-crop samples are shown in table I.

The variations in total sugar content of 49 samples of the 1933 crop are shown in table II.

In both years the total sugar content of samples from inside districts was distinctly higher than in samples from outside districts. The maximum values for outside districts rarely exceeded the minimum for inside districts.

The data on sucrose and on dextrose-levulose ratio are given in tables III and IV.

Neither the sucrose content nor the dextrose-levulose ratio appears to have any relation to district of origin or to size. It is of interest that dextrose greatly exceeds levulose in prunes.

Grateful acknowledgment is made of the assistance of R. D. BETHEL and F. FILIPELLO in this work, and to the United Prune Growers for samples.—
PAUL F. NICHOLS, *University of California, Berkeley, Calif.*

LITERATURE CITED

1. GALE, L., and CRUESS, W. V. Composition of dried California prunes of the French (prune d'Agen) variety. Fruit Prod. Jour. and Amer. Vinegar Ind. **10**: 302–304. 1931.
2. HILTNER, R. S., and HATHERELL, B. L. Unpublished report to the Dried Fruit Association of California. 1928.
3. LOTHROP, R. E., and HOLMES, R. L. Determination of dextrose and levulose in honey by use of iodine-oxidation method. Ind. & Eng. Chem. Anal. Ed. **3**: 334–339. 1931.
4. MRAK, E. M., SMITH, C., and HENRIQUES, V. The carbohydrates of dried California French prunes (prune d'Agen). Amer. Soc. Hort. Sci. Proc. **30**: 238–242. 1933.
5. NICHOLS, P. F., and REED, H. M. Relation of specific gravity to the quality of dried prunes. Hilgardia **6**: 561–583. 1932.
6. SHAFFER, P. A., and HARTMANN, A. F. The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. **45**: 365–390. 1921.

ISOLATION AND DETERMINATION OF STARCH IN PLANT TISSUE

The removal of starch from plant tissues by dispersion in various reagents has often been employed in the quantitative estimation of this substance (**5**). Recently DENNY (**1, 2**) and HOPKINS (**3**) have reviewed those methods wherein mineral acids or salt solutions are used as the dispersing agents. We have found that hot dilute ethanol can also be used for this purpose after the plant tissue has been suitably pretreated with an acid-alcohol reagent. In the course of a microchemical study on the reserve carbohydrates of apple wood spurs, it was noticed that after the tissue had been treated with a boiling solution of 1 per cent. nitric acid in 85 per cent. ethanol the starch granules could be dispersed in boiling 20 per cent. ethanol. A procedure employing this neutral and salt free extraction has been developed. Its essential features are presented below:

0.500 to 2.500-gm. samples (depending upon the anticipated starch content) are weighed into 40-cc. alundum crucibles. The latter are placed in a Soxhlet extractor and extracted for 24 hours with a 2:1 benzene-ethanol mixture. The crucibles are then taken from the extractor and the major portion of the solvent removed by suction. The contents of a crucible are transferred to a 300-cc. flask and covered with 180 cc. of the ethanolic-nitric acid reagent prepared by diluting 10.65 cc. of concentrated nitric acid, sp. gr. 1.40, to 1 liter with 85.1 per cent. ethanol by volume. This mixture is then boiled under reflux for 30 minutes. The residue remaining in the flask is now recovered by filtration through the original alundum crucible and washed free of acid with 75–90 cc. of hot 95 per cent. ethanol. The residue is partially dried by suction and again introduced into a 300-cc. flask. 100 cc. of 20 per cent. ethanol are then added and the contents of the flask boiled under reflux for 20–25 minutes. The insoluble matter left in the flask is now removed by filtration and washed with 40–50 cc. of boiling 20 per cent. ethanol. The filtrate and washings are combined. This solution contains the starch polysaccharides present in the original tissue.

If it is desired to isolate the starch polysaccharides the 20 per cent. ethanol extract is concentrated *in vacuo* to about 25 to 30 cc. This concentrate is poured, with stirring, into 10 volumes of a 1:1 acetone-ethanol mixture, thereby precipitating the polysaccharides which are then recovered by centrifugation and filtration.

For the estimation of the starch content of the original tissue the 20 per cent. ethanol extract is evaporated on a hot plate to a small volume (10 cc.) and 100 cc. of 2.5 per cent. hydrochloric acid added. The solution is then boiled under reflux for 2.5 hours, cooled, neutralized, and made to volume.

Glucose is determined on an aliquot portion and expressed as starch in the usual manner.

It has been observed that with certain types of plant tissue, the 20 per cent. ethanol extract contains in addition to the starch polysaccharides some non-starch polysaccharides. In such a case, a fractionation of the extract by a method such as proposed by SMALL (**4**) is recommended before proceeding with either the isolation of the polysaccharide from the extract by precipitation with the acetone-ethanol mixture, or the hydrolysis of the extract to reducing sugars.—CARL NIEMANN, R. H. ROBERTS, AND KARL PAUL LINK, *Biochemistry Research Laboratory, Department of Agricultural Chemistry and Department of Horticulture, University of Wisconsin, Madison, Wisconsin.*

LITERATURE CITED

1. DENNY, F. E. Improvements in methods of determining starch in plant tissue. Contrib. Boyce Thompson Inst. **6**: 129–146. 1934.
2. ————————. Starch determination methods involving solubility in acids. Contrib. Boyce Thompson Inst. **6**: 381–393. 1934.
3. HOPKINS, C. Y. Polarimetric estimation of starch. Canadian Jour. Res. **11**: 751–758. 1934.
4. SMALL, J. C. Quantitative determination of soluble starch in the presence of starch and its hydrolytic cleavage products. Jour. Amer. Chem. Soc. **41**: 107–120. 1919.
5. WALTON, R. P. A comprehensive survey of starch chemistry. Chem. Catalog Co., New York. 1928.

NOTES

Annual Election.—The annual election of the American Society of Plant Physiologists held during the last quarter of the fiscal year has resulted in the election of Dr. A. E. MURNEEK, former secretary-treasurer, as president for 1935–1936. The new vice-president is Dr. D. R. HOAGLAND, University of California; and the new secretary-treasurer is Dr. W. F. LOEHWING, University of Iowa. The results of the balloting insure a continuation of the policies and ideals of the preceding administration which have done much to strengthen the foundations of the Society and to insure its continuous progress. Newly elected officers always appreciate the spirit of cordial cooperation on the part of members. Such cooperative effort will be especially helpful in connection with the annual meetings and programs to be presented at St. Louis in December.

Minneapolis Meeting.—The summer meetings of the Society at Minneapolis and St. Paul were very successful as measured by the criteria of attendance and interest. With the exception of the vice-presidents, the officers of the Society for 1934–1935 and 1935–1936 were present. Almost 80 members and friends attended the sessions, and great interest was shown in the various papers presented. One of the best attended programs was the symposium on seed dormancy and related problems.

About 40 members were present Wednesday at the picnic dinner at the summer home of Dr. and Mrs. R. B. Harvey on the St. Croix river. This was a most delightful social occasion, no one being allowed to be either hungry or thirsty. The visitors had the unique privilege of enjoying Dr. HARVEY'S fine portrait gallery of famous plant physiologists. Music and merriment added zest at the close of the picnic. The generous and cordial hospitality of the hosts was much appreciated by everyone.

St. Louis Meeting.—Preparations for the St. Louis meeting are already in progress. It is hoped that early action may be taken on all important matters concerned with accommodations and arrangements in order that appropriate announcements may be made in the October number of PLANT PHYSIOLOGY.

It would be very helpful in preparing programs if the proposed titles could be in the hands of the program committee early. Any symposia to be organized should be arranged at the earliest possible moment, so that participants may have as much time as may be needed to prepare their contributions. Undue haste, necessitated by short time, usually lowers the value of contributions. Would it be possible to select papers from among those offered, and confine programs to a smaller number of papers, and to a reason-

able length of time? Papers which could not be accommodated with time for personal presentation might be presented as mimeographed abstracts, with opportunity for interested readers to ask questions and elaboration if desired.

These suggestions may not be practicable; but it does seem that program committees should recognize the growing need for some way to relieve the current overloading of the programs. This arises out of the effort to accommodate everyone, which has always seemed to be desirably democratic. Another way out would be to practice close segregation of related material and provide for two or more simultaneous programs in adjacent or nearby rooms.

New England Section.—A very enthusiastic report of the second annual meeting of the New England section has been received from the secretary, Dr. LINUS H. JONES, Massachusetts. The meeting was held at the University of New Hampshire, Durham, May 17–18. The officers elected for the coming year are as follows: chairman, Dr. T. G. PHILLIPS, New Hampshire; vice-chairman, Dr. B. E. GILBERT, Rhode Island; secretary-treasurer, Dr. LINUS H. JONES, Massachusetts.

The meeting was attended by men from a larger number of institutions than was the first meeting in 1934. It was voted to meet at Kingston, Rhode Island next year.

In order to emphasize the value of these regional meetings, the titles of papers presented before the gathering are presented here.

Seedling culture in sand. A. A. DUNLAP, Connecticut.

Carbon dioxide in the forest. H. I. BALDWIN, Research Forest, Hillsboro, New Hampshire.

The effect of simultaneously varied radiation, nitrogen, and potassium on the growth of white pine seedlings. R. R. GAST, Harvard.

Micro determinations of nitrogen, phosphorus, and potassium in plant material. E. A. SNOW, Harvard.

Nitrogen, phosphorus, and potassium contents of foliage of fertilized northern white pine plantations. W. H. CUMMINGS, Harvard.

Plant transpiration as modified by potassium. A. G. SNOW, Yale.

The cumulative effect of 25 years of liming with calcic vs. magnesic liming materials on the mineral nutrient composition of the edible portions of plants. D. R. WILLARD, Rhode Island.

The present status of legal recognition of the potential acidity or alkalinity of fertilizers. J. B. SMITH, Rhode Island.

Testing plants with diphenylamine. L. H. JONES, Massachusetts.

Assimilation of nitrogen by the tomato plant. H. E. CLARK, Yale.

The effect of heavy fertilization with ammonia on the glutamine content of beets. H. B. VICKERY, Connecticut.

The quantities of non-glucose reducing substances in plant juices. F. S. SCHLENKER, Rhode Island.

The determination of starch in plant tissue. G. W. PUCHER, Connecticut.

The nature of winter injury in apple trees. F. H. STEINMETZ, Maine.

Growth hormones in plants. E. A. NAVEZ, Harvard.

Experiments in highbush blueberry culture. J. S. BAILEY, Massachusetts.

The physical basis of mycotrophy in *Pinus*. A. B. HATCH, Harvard.

Ethylene induced epinasty. C. G. DEUBER, Yale.

The determination and recording of light intensities. R. H. WALLACE, Connecticut.

Respiratory systems of *Lupinus albus*. F. N. CRAIG, Harvard.

Purdue Section.—The Purdue Section reports another good series of meetings during the past year. The attendance has ranged from 20 to 35, with an average of 25. Dr. J. H. MCGILLIVRAY was president of the section during 1934–1935, A. T. GUARD secretary-treasurer, and Dr. C. L. PORTER chairman of the program committee. The titles of papers and addresses presented are as follows:

October 15, 1934, Ancient cornfields of America. PAUL WEATHERWAX, Indiana University, guest speaker.

November 5, Reproduction in fungi. G. B. CUMMINS.

November 19, Special problems of fungus nutrition. R. B. BAINS.

December 3, Reproduction in flowering plants. A. T. GUARD.

December 17, Plant hormones. D. M. DOTY.

January 21, 1935, Reports of the A. A. A. S. meetings.

February 4, Relationships existing between the functions and structures of plants. E. J. KOHL.

February 18, Present status of mineral nutrition. S. F. THORNTON.

March 4, Nitrogen metabolism. H. R. KRAYBILL.

March 18, Soil types and plant growth. S. D. CONNER.

April 1, Practical applications of fermentation phenomena. P. A. TETRAULT.

April 15, Modern trends in plant physiology. R. E. GIRTON.

The meeting on April 15 closed the activities of the year, the address closing the annual dinner festivities. The officers elected for the ensuing year are J. T. SULLIVAN, president; R. B. ZUMSTEIN, secretary-treasurer; and E. C. STAIR, chairman of the program committee.

Chemical Methods Reprints.—Reprints of the supplementary report of the chemical methods committee may be obtained from the chairman of the committee, Dr. W. E. TOTTINGHAM, Agricultural Chemistry Building,

University of Wisconsin, Madison, Wisconsin. The price authorized for these reprints is 15 cents per copy post free. Lots of 8 ordered at one time may be obtained for $1.00 post free. Laboratories with groups of students will find this club rate economical.

The original reports published some years ago can no longer be supplied in complete sets, as some of the parts are exhausted. There are still available 4 copies of the section on soluble carbohydrates, and 38 copies of the section on peptides and basic forms of nitrogen. These also are held by Dr. TOTTINGHAM, who will furnish them as long as the few remaining sections last.

Hugo de Vries.—With the death of HUGO DE VRIES at Lunteren, Holland, on May 21, 1935, biological science has lost one of its most revered and beloved leaders. Born February 16, 1848, his life spanned the entire period of modern biological thought. To the development of experimental biology he brought a rich experience in the dramatic development of biological speculative philosophy which occurred during the several decades following the publication of the Origin of Species. He was a leader in the movement from speculative to experimental work during the last quarter of the 19th century. His great achievements in plant physiology and genetics will stand as an enduring monument to his genius. To us and to future generations of biologists his life will stand as an inspiration and a challenge to emulate his example of patient industry and creative thinking. His name will always stand high among the illustrious biological philosophers of the late 19th and early 20th century period.

Friedrich August Ferdinand Christian Went.—On July 26, 1935, Professor WENT, for many years professor of Botany at the University of Utrecht, passed away. He was elected president of the Sixth International Botanical Congress which is to convene at Amsterdam early in September, and will be greatly missed during the deliberations of the congress. He has been a great leader in the study of plant responses, and especially in the field of hormones. He was a corresponding member of the American Society of Plant Physiologists. His passing will be deeply mourned by all plant physiologists.

Annual Review of Biochemistry.—Volume IV of the Annual Review of Biochemistry has just been issued by the Stanford University Press. It maintains the high standards set by the preceding volumes, and does great credit to the editors of the series, to the reviewers, and to the publishers. In the few years since this annual review was started, it has become an indispensable guide to the rapid developments in this expanding field of research.

The current volume contains 27 reviews, a number of which will be especially useful to plant physiologists. Among these are mentioned the sections on permeability, biological oxidations and reductions, enzymes, plant pigments, the alkaloids, mineral nutrition of plants, growth substances in plants, chemistry of bacteria, etc.

The reviewers have a very difficult task to perform, since they must choose those papers for review that in their judgment contribute most seriously to the advancement in the general field covered. The sifting and winnowing of the far flung literature that is produced each year is no mean task in any field; and the provision of a reliable guide to the currents of advancement requires clear vision and sound judgment. The writer of this note feels that the great majority of the reviewers have done unusually well with their assignments.

The series is issued in handsome binding, and the press work is uniformly good. The price of volume IV is the same as for the preceding annual volumes, $5.00 per copy. The series constitutes a valuable history of the development of biochemistry in our times. Orders for this or any of the earlier volumes may be sent to the Stanford University Press.

Temperature and Living Matter.—The eighth volume of the Protoplasma-Monographien published by Gebrüder Borntraeger, Berlin, is entitled Temperature and Living Matter. The author is JAN BĚLEHRÁDEK, Professor of General Biogology, Caroline University, Prague. It is written in English, and will therefore be more readily available to American students. It contains eleven chapters with titles as follows: general principles of biological temperature action; rate of biological processes at biokinetic temperatures; variation of temperature coefficients with external and internal factors; theories of temperature coefficients; chemical properties of living systems at biokinetic temperatures; variations of morphological equilibria at biokinetic temperatures; physical properties of living systems at biokinetic temperatures; freezing and frost resistance; chilling, chill coma, and death by chilling; injury by heat and heat resistance; and stimulative effects of temperature.

There are 229 pages of text, 37 pages of bibliography and author index, and 11 pages of subject index. The work is written in simple, direct style that will be appreciated by busy readers. The ground is well enough covered on the animal side, not quite so well on the plant side. It is frequently the case that general biology is mainly animal biology in general terms. It presents a good summary of the general effects of temperature on living protoplasm.

The price quoted for this volume, RM 18, is for cloth binding uniform to the series.